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SEÑOR**SUPERINTENDENTE DE INDUSTRIA Y COMERCIO****E.****S.****D.****REF:****Expediente 08 075.084****TÍTULO SOLICITADO:****MÉTODOS PARA TRATAR INFECCIONES DE
TRACTO GASTROINTESTINAL CON TIGECICLINA.****PUBLICACIÓN:****Gaceta: 597 de 31 de Octubre 2008, N.P. 943.****PRIORIDAD:****US 60/753,161 de 22/12/2005.****SOLICITANTE:****WYETH.****APODERADO:****ALVARO CORREA ORDOÑEZ****TRÁMITE:****SUSTENTACION DE OPOSICIÓN**

JOSÉ LUIS REYES VILLAMIZAR, mayor de edad, identificado como aparece al pie de mi firma, abogado en ejercicio, titular de la tarjeta profesional número 44655 del Consejo Superior de la Judicatura, obrando en calidad de apoderado de la sociedad **LABORATORIO FRANCO-COLOMBIANO, LAFRANCOL S.A.**, dentro del trámite de la referencia, en ejercicio de lo dispuesto por el artículo 42 inciso 2º de la Decisión 486 de 2000, por medio del presente escrito, procedo a sustentar y complementar la oposición presentada el 02 de Febrero de 2009, en los siguientes términos:

I. RATIFICACIÓN

Comedidamente me permito presentar los argumentos planteados en la oposición referida, los cuales, como se dijo en su momento, demuestran que la solicitud de la referencia carece de los requisitos de patentabilidad establecidos por los artículos 14, 16, 18 y 20 literal d) de la Decisión 486 y que, como tal, no merece recibir el beneficio patentario.

II. COMPLEMENTO DE INFORMACIÓN

En primer lugar, y estando ante la revelación textual de un método de tratamiento con Tigeciclina para una infección gastrointestinal, específicamente infecciones del tipo colitis pseudomembranosa asociada a antibióticos provocada por *C. difficile* y enterocolitis provocada por *S. aureus* y cepas resistentes a meticilina; es relevante señalar que dichas revelaciones, explícitamente mencionadas en todo el capítulo reivindicatorio de la solicitud en cuestión, son inaceptables a la luz de la Decisión 486.

En efecto, a la luz de la normativa subregional resulta inaceptable pretender atribuirse exclusiva sobre un método de tratamiento, ya que como lo expresa literalmente la Decisión 486 de la Comunidad Andina de Naciones en su aparte d) del artículo 20 del capítulo I, acerca de los requisitos de patentabilidad, *“no serán patentables los métodos terapéuticos o quirúrgicos para el tratamiento humano o animal, así como los métodos de diagnóstico aplicados a los seres humanos o a animales”*, y si tenemos en cuenta que la solicitud en cuestión expone en todas sus reivindicaciones un método de tratamiento, queda claramente expuesta la improcedencia directa para otorgar el privilegio de patente de invención a esta solicitud.

En un segundo aspecto, y esta vez refiriéndonos a la carencia de nivel inventivo, es relevante señalar que las composiciones farmacéuticas que describe el capítulo reivindicatorio de la solicitud colombiana en trámite, específicamente cuando se refiere a *“la composición farmacéutica está en forma sólida”* (reivindicación 11), a *“la composición farmacéutica de Tigeciclina que tiene al menos un recubrimiento entérico”* (reivindicación 13) y a *“gránulos de múltiples partículas, con revestimiento entérico”* (reivindicación 23); corresponden a características de formulaciones que son reconocidas por cualquier persona medianamente versada en la materia, y que así mismo se encuentran lo suficientemente descritas en el estado de la técnica.

Es así como dentro de la diferenciación de las formulaciones sólidas para administración oral, tales como lo son los comprimidos, existen dentro de dichos comprimidos unos que se pueden denominar como “comprimidos especiales”,

dado el hecho que presentan alguna(s) característica(s) fármacotécnica(s) diferente(s) a las descritas para los comprimidos convencionales. Dentro de los “comprimidos especiales” los de mayor utilización pueden ser los sublinguales, orodispersables, masticables, efervescentes, multicapas, vaginales, de implantación, de liberación controlada y recubiertos¹. Dentro de estas dos últimas categorías se encontrarían relacionadas las características de las formulaciones que se describen como *“la composición farmacéutica está en forma sólida”*, *“la composición farmacéutica de Tegiciclina que tiene al menos un recubrimiento entérico”* y *“gránulos de múltiples partículas, con revestimiento entérico”*; que menciona la solicitud colombiana en cuestión, ya que corresponden sin duda alguna a gránulos y/o tabletas que liberan el principio activo de manera controlada (mas exactamente localizada) gracias a un recubrimiento gastrorresistente o entérico.

Como cualquier persona medianamente versada en la materia reconoce, un recubrimiento gastrorresistente o entérico, consiste en la aplicación de materiales en la superficie del núcleo o formulación inicial a recubrir, que garanticen la integridad de la cubierta a su paso por el estómago, manteniéndose impermeables e insolubles al pH gástrico y durante el tiempo correspondiente de transito en el mismo. Como materiales de recubrimiento entérico se emplean, habitualmente, polímeros de naturaleza ácida, que en función de su pK y del pH del medio, se mantendrán no disociados (poco solubles) o se disociaran (solubles). Entre los mas utilizados se encuentran el acetofalato de celulosa, el acetofalato de polivinilo, los copolímeros del ácido metacrílico (Eudagrits), la goma laca, el ftalato de hidroxipropilmetilcelulosa, entre otros, y para conseguir el perfil de liberación adecuado se suele recurrir a la mezcla de diferentes agentes filminógenos².

Como se puede apreciar, específicamente en la reivindicación 14 de la solicitud colombiana en trámite, los compuestos que se describen como parte del revestimiento entérico de dichas formulaciones, corresponden a las características anteriormente mencionadas para las sustancias convencionalmente útiles como agentes de recubrimiento entérico, e inclusive se hace mención explícita del acetofalato de celulosa, de los copolímeros del ácido metacrílico y del ftalato de

¹ Vila Jato J.L. Tecnología Farmacéutica Volumen II, página 142.

² Vila Jato J.L. Tecnología Farmacéutica Volumen II, página 154.

hidroxipropilmetilcelulosa, que como vimos, son algunos de los compuestos más comunes para la aplicación de este tipo de recubrimientos.

En otras palabras, puede afirmarse que la inclusión de Tigeciclina en este tipo de formulaciones farmacéuticas, no sustenta de ninguna manera nivel inventivo alguno, puesto que dicha inclusión corresponde a la aplicación rutinaria e implícita, de los correspondientes ensayos de preformulación y formulación, que se requieren cuando cualquier principio activo, junto a unos excipientes, se formulan para obtener un medicamento con las características de seguridad, efectividad, eficacia y estabilidad deseadas. Esta misma argumentación aplica para las formulaciones farmacéuticas mencionadas en la reivindicaciones 6, 7, 10, 12, y 16 de la solicitud colombiana en cuestión, específicamente en lo referido a tabletas, cápsulas, píldoras, polvos y polvos liofilizados, así como formulaciones líquidas tipo solución o suspensión, puesto que estas formulaciones son mucho menos complejas y al mismo tiempo son reconocidas por cualquier persona medianamente versada en la materia y los métodos para su obtención se encuentran lo suficientemente descritos en el estado de la técnica.

Cabe también señalar, que la solicitud en cuestión, específicamente en su reivindicación 12, revela una incongruencia al exponer que: *“Método de conformidad con la reivindicación 11, caracterizado porque la forma líquida se elige de tabletas, capsulas, polvos y tortas y polvos liofilizados”* puesto que, en primer lugar, la reivindicación 11 a la que se refiere menciona que la formulación farmacéutica es a en forma sólida, y en segundo lugar porque obviamente las tabletas, cápsulas, polvos y tortas y polvos liofilizados no corresponden a la clasificación de formulaciones farmacéuticas líquidas.

En otro aspecto, pero refiriéndonos aun a la carencia de nivel inventivo, existen documentos que demuestran la anterioridad de la utilidad de la Tegiciclina en el tratamiento de los diversos tipos de infecciones gastrointestinales que se mencionan en la solicitud colombiana en trámite, veamos:

In Vitro And In Vivo Antibacterial Activities Of A Novel Glycylcycline, The 9-T-Butylglycylamido Derivative Of Minocycline (GAR-936)³.

Abstract: The 9-t-butylglycylamido derivative of minocycline (TBG-MINO) is a recently synthesized member of a novel group of antibiotics, the glycylcyclines. This new derivative, like the first glycylcyclines, the N,N-dimethylglycylamido derivative of minocycline and 6-demethyl-6-deoxytetracycline, possesses activity against bacterial isolates containing the two major determinants responsible for tetracycline resistance: ribosomal protection and active efflux. The in vitro activities of TBG-MINO and the comparative agents were evaluated against strains with characterized tetracycline resistance as well as a spectrum of recent clinical aerobic and anaerobic gram-positive and gram-negative bacteria. TBG-MINO, with an MIC range of 0.25 to 0.5 microgram/ml, showed good activity against strains expressing tet(M) (ribosomal protection), tet(A), tet(B), tet(C), tet(D), and tet(K) (efflux resistance determinants). TBG-MINO exhibited similar activity against methicillin-resistant Staphylococcus aureus (MRSA), penicillin-resistant streptococci, and vancomycin-resistant enterococci (MICs at which 90% of strains are inhibited, < or = 0.5 microgram/ml). TBG-MINO exhibited activity against a wide diversity of gram-negative aerobic and anaerobic bacteria, most of which were less susceptible to tetracycline and minocycline. The in vivo protective effects of TBG-MINO were examined against acute lethal infections in mice caused by Escherichia coli, S. aureus, and Streptococcus pneumoniae isolates. TBG-MINO, administered intravenously, demonstrated efficacy against infections caused by S. aureus including MRSA strains and strains containing tet(K) or tet(M) resistance determinants (median effective doses [ED50s], 0.79 to 2.3 mg/kg of body weight). TBG-MINO demonstrated efficacy against infections caused by tetracycline-sensitive E. coli strains as well as E. coli strains containing either tet(M) or the efflux determinant tet(A), tet(B), or tet(C) (ED50s, 1.5 to 3.5 mg/kg). Overall, TBG-MINO shows antibacterial activity against a wide spectrum of gram-positive and gram-negative aerobic and anaerobic bacteria including strains resistant to other chemotherapeutic agents. The in vivo protective effects, especially against infections caused by resistant bacteria, corresponded with the in vitro activity of TBG-MINO.

In Vitro Activities of Tigecycline (GAR-936) against Multidrug-Resistant Staphylococcus aureus and Streptococcus pneumoniae⁴.

Abstract: During recent years, a dramatic increase in bacterial resistance to antibiotics has been observed worldwide, both in community and nosocomial isolates, curtailing the use of many valuable antibiotics. In France, more than 50% of Streptococcus pneumoniae strains isolated in the community are resistant to penicillin and macrolides (8), and more than 40% of Staphylococcus aureus strains isolated in hospitals are resistant to β -lactams, aminoglycosides, macrolides, and fluoroquinolones, including the newer compounds ($\approx$ 100% of the

³ Petersen PJ, Jacobus NV, Weiss WJ, Sum PE, Testa RT. Antimicrob Agents Chemother. 1999 Apr;43(4):738-44.

⁴ M. D. Kitzis, A. Ly, F. W. Goldstein. ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Jan. 2004, p. 366-367 Vol. 48, No. 10066-4804/04/\$08.0010 DOI: 10.1128/AAC.48.1.366-367.2004 Copyright © 2004, American Society for Microbiology.

methicillin-resistant *S. aureus* [MRSA] strains) (5). *S. aureus* strains with low-level resistance to glycopeptides have been isolated in our hospital for more than 10 years (6), and resistance to quinupristin-dalfopristin (Synercid) is not uncommon due to the frequent use of oral pristinamycin in France. Most of these multidrug-resistant (MDR) bacteria are resistant to tetracyclines, emphasizing the need for new compounds. Tigecycline (GAR-936) is the 9-t-butylglycylamino derivative of minocycline, a new generation of tetracyclines called glycylcyclines. These glycylcyclines overcome tetracycline resistance due to both ribosomal protection and efflux determinants (1–3, 7). The aim of this study was to assess the in vitro activity of GAR-936 against MDR *S. pneumoniae* and *S. aureus* in comparison with tetracycline, minocycline, telithromycin, linezolid, and quinupristin-dalfopristin.

In Vitro Activity Of Tigecycline Against 6792 Gram-Negative And Gram-Positive Clinical Isolates From The Global Tigecycline Evaluation And Surveillance Trial (TEST Program, 2004)⁵.

Abstract: Tigecycline, a new glycylcycline antibiotic, has shown promising in vitro activity against many common pathogens, including multidrug-resistant strains. To determine the activity of tigecycline against a broad range of pathogens from diverse populations and geographic areas, the Tigecycline Evaluation and Surveillance Trial (TEST Program) commenced in 2003. This study evaluated the activity of tigecycline and commonly used antimicrobials against 6792 clinical isolates from 40 study centers in 11 countries. Tigecycline was the most active agent tested against Gram-positive facultative species including multidrug-resistant strains. MIC₉₀ results (microg/mL) for tigecycline against *Enterococcus faecalis*, *Enterococcus faecium*, *Staphylococcus aureus*, *Streptococcus agalactiae*, and *Streptococcus pneumoniae* were 0.12, 0.12, 0.25, and 0.25 microg/mL, respectively. Tigecycline was active against *Enterobacteriaceae* with an MIC₉₀ of 1 microg/mL. *Haemophilus influenzae* was very susceptible to tigecycline with an MIC₉₀ of only 0.25 microg/mL. *Pseudomonas aeruginosa* was the least susceptible organism tested against tigecycline. Tigecycline appears to be a promising new glycylcycline agent for the treatment of many types of pathogens with varying resistance phenotypes.

Antimicrobial Activity Of Tigecycline (GAR-936) Tested Against 3498 Recent Isolates Of *Staphylococcus Aureus* Recovered From Nosocomial And Community-Acquired Infections⁶.

Abstract: Tigecycline is a novel 9-t-butylglycylamido derivative of minocycline that has demonstrated activity against a variety of Gram-positive and-negative bacterial pathogens. In vitro activity of tigecycline and comparator agents was determined for 3498 recent (2000-2003) strains of *Staphylococcus aureus* recovered from patients with either nosocomial or community-acquired infections. Oxacillin-susceptible and-resistant *S. aureus* from both patient populations displayed

⁵ Hoban DJ, Bouchillon SK, Johnson BM, Johnson JL, Dowzicky MJ; Tigecycline Evaluation and Surveillance Trial (TEST Program) Group. *Diagn Microbiol Infect Dis.* 2005 Jul;52(3):215-27.

⁶ Fritsche, TR | Jones, RN. *International Journal of Antimicrobial Agents* [Int. J. Antimicrob. Agents]. Vol. 24, no. 6, pp. 567-571. Dec 2004.

identical results for tigecycline (MIC sub(50) and MIC sub(90) results at 0.25 and 0.5 mg/L, respectively) and all strains were inhibited by 1 mg/L or less. While co-resistances to other antimicrobial classes were present in oxacillin-resistant strains, susceptibility to tigecycline remained unaffected, making the compound an attractive candidate for treatment of serious hospital as well as community-acquired staphylococcal infections.

Como se puede apreciar en todas las anteriores publicaciones de carácter científico, la utilidad de Tigeciclina en el tratamiento de infecciones producidas por bacterias anaerobias, específicamente cepas de *S. aureus* resistentes a meticilina es ampliamente reconocida, y por lo tanto resulta mas que evidente para una persona medianamente versada en la materia, incluir y utilizar Tegiciclina a través de formulaciones farmacéuticas, para el tratamiento de infecciones como las que reivindica la solicitud colombiana.

La publicación internacional WO 2004/017925 publicada el 04 de Marzo de 2004 y titulada "METHODS AND REAGENTS FOR PREVENTING BACTEREMIAS" revela:

b) orally administering to said patient one or more antibiotics selected from the group consisting of: teicoplanin, daptomycin, oritavancin, dalbavancin, everninomycin, virginiamycin, quinupristin, dalbopristin, linezolid, tigecycline, pristnamycin, nisin, moenomycin, gemifloxacin, tunicamycin, cinnamycin, laspartomycin, novobiocin, ciprofloxacin, moxifloxacin, chloramphenicol, nitrofurantoin, cyclo-(Leu-Pro), fosfomycin, telithromycin, azithromycin, magainin, iseganan, BMS-284,756, L-749,345, ER-35,786, S-4661, L-786,392, MC-02479, Pep5, RP 59500, and TD-6424 in an amount and for a duration sufficient to substantially decolonize the intestinal tract of said patient of said bacteria.

(...)

7. The method of any of claims 1, 2, or 5, wherein said bacteria are antibiotic-resistant.

(...)

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13. The method of claim 12, wherein said bacteria is *S. aureus*, *S. epidermidis*, *S. hominis*, *S. saprophyticus*, *S. hemolyticus*, *S. capitis*, *S. auricularis*, *S. lugdenis*, *S. warneri*, *S. saccharolyticus*, *S. caprae*, *S. pasteurii*, *S. schleiferi*, *S. xylosus*, *S. cohnii*, or *S. simulans*.

(...)

23. The method of any of claims 1, 2, or 5, wherein said patient has or is at risk for enteritis, colitis, or mucositis of the intestinal tract.

La publicación internacional WO 2004/096143 publicada el 11 de Noviembre de 2004 y titulada "METHODS FOR REDUCING OR PREVENTING TRANSMISSION OF NOSOCOMIAL PATHOGENS IN A HEALTH CARE FACILITY" revela:

1. A method for reducing or preventing the transmission of a nosocomial pathogen, said method comprising the steps of:
 - a. identifying a carrier who is colonized with a nosocomial pathogen; and
 - b. administering an antibiotic, in an amount and for a duration sufficient to prevent colonization or infection by said pathogen, to a population of individuals at risk of being colonized or infected by said pathogen from said carrier.

(...)

2. The method of claim 1, wherein the gastrointestinal tract of said carrier is colonized with said nosocomial pathogen and said antibiotic is orally administered to said carrier.

(...)

7. The method of claim 1, wherein said antibiotic is selected from the group consisting of teicoplanin, daptomycin, oritavancin, dalbavancin, everninomycin, quinupristin/dalfopristin, linezolid, tigecycline, colistin, amphotericin, nystatin, iseganan, and ramoplanin.

(...)

27. The method of claim 26, wherein said bacterium is *S. aureus*, *S. epidermidis*, *S. hominis*, *S. saprophyticus*, *S. hemolyticus*, *S. capitis*, *S. auricularis*, *S. lugdenis*, *S. warneri*, *S. saccharolyticus*, *S. caprae*, *S. pasteurii*, *S. schleiferi*, *S. xylosus*, *S. cohnii*, or *S. simulans*.

(...)

28. The method of claim 26, wherein said bacterium is resistant to methicillin.

(...)

34. The method of claim 21, wherein said bacterium is *Clostridium difficile* or *Clostridium perfringens*.

La publicación internacional WO 2005/023263 publicada el 17 de Marzo de 2005 y titulada "USE OF TIGECYCLINE, ALONE, OR IN COMBINATION WITH RIFAMPIN TO TREAT OSTEOMYELITIS AND/OR SEPTIC ARTHRITIS" revela:

1. A method of treating an infection in bone or bone marrow in a mammal comprising administering to the mammal a pharmacologically effective amount of tigecycline.

(...)

7. The method of claim 6 wherein the infection is comprised of *Neisseria meningitidis*, *Mycobacterium tuberculosis*, *Mycobacterium leprae*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, or *Haemophilus influenzae*.

(...)

9. The method of claim 8 where the antibiotic resistance is selected from the group consisting of methicillin resistance, glycopeptide resistance, tetracycline resistance, oxytetracycline resistance, doxycycline resistance; chlortetracycline resistance, minocycline resistance minocycline resistance, glycylcycline resistance, cephalosporin resistance, ciprofloxacin resistance, nitrofurantoin resistance, trimethoprim-sulfa

La patente americana US 2004/0106590 publicada el 03 de Junio de 2004 y titulada "METHODS AND REGENTS FOR TREATING INFECTIONS OF CLOSTRIDIUM DIFFICILE AND DISEASES ASSOCIATED THEREWITH" revela:

1. A method for treating a subject having an infection of *Clostridium difficile* or preventing an infection of *Clostridium difficile* in said subject, said method comprising administering to said subject an effective amount of rifalazil.

(...)

13. The method of claim 1, further comprising administering to said subject one or more antibiotics selected from the group consisting of β -lactams, β -lactamase inhibitors, aminoglycosides, tetracyclines, lipopeptides, macrolides, ketolides, lincosamides, streptogramins, sulphonamides, oxazolidinones, quinolones, rifamycins, glycopeptides, metronidazole, garenoxacin, ramoplanin, faropenem, polymyxin, tigecycline, AZD2563, and trimethoprim.

Como se puede apreciar en estas tres anterioridades, se menciona la utilidad de la Tigeciclina en el tratamiento de infecciones relacionadas a cepas de *S. aureus*, cepas resistentes a la meticilina y cepas de *C. difficile*, así como en el tratamiento de colitis.

En conclusión, la solicitud colombiana en trámite carece de los requisitos fundamentales para otorgársele el privilegio de patente de invención, en primer lugar, porque incurre en una improcedencia directa de patentabilidad al reivindicar métodos terapéuticos relacionados a Tigeciclina; y en segundo lugar porque carece absolutamente de nivel inventivo al formular Tigeciclina en formulaciones

tipo gránulos o tabletas con recubrimiento entérico, para el tratamiento de diversos tipos de infecciones relacionadas a dicho uso, en donde ambos temas se encuentran lo suficientemente descritos en el estado de la técnica.

Por todo lo anterior, le solicito Señor Superintendente, que analice los argumentos citados con el rigor y detenimiento habituales y que decrete, además de la negación de la solicitud en comento, el archivo del citado expediente.

III. ANEXOS

- Vila Jato J.L. Tecnología Farmacéutica Volumen II, páginas 142 y 154.
- Abstracts de todas las publicaciones científicas mencionadas en el acápite de Complemento de Información.
- La publicación internacional WO 2004/017925 publicada el 04 de Marzo de 2004 y titulada "METHODS AND REAGENTS FOR PREVENTING BACTEREMIAS" capítulo reivindicatorio.
- La publicación internacional WO 2004/096143 publicada el 11 de Noviembre de 2004 y titulada "METHODS FOR REDUCING OR PREVENTING TRANSMISSION OF NOSOCOMIAL PATHOGENS IN A HEALTH CARE FACILITY" capítulo reivindicatorio.
- La publicación internacional WO 2005/023263 publicada el 17 de Marzo de 2005 y titulada "USE OF TIGECYCLINE, ALONE, OR IN COMBINATION WITH RIFAMPIN TO TREAT OSTEOMYELITIS AND/OR SEPTIC ARTHRITIS" capítulo reivindicatorio.
- La patente americana US 2004/0106590 publicada el 03 de Junio de 2004 y titulada "METHODS AND REGENTS FOR TREATING INFECTIONS OF CLOSTRIDIUM DIFFICILE AND DISEASES ASSOCIATED THEREWITH" capítulo reivindicatorio.

Señor Superintendente

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In Vitro and In Vivo Antibacterial Activities of a Novel Glycylcycline, the 9-*t*-Butylglycylamido Derivative of Minocycline (GAR-936)

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The 9-*t*-butylglycylamido derivative of minocycline (TBG-MINO) is a recently synthesized member of a novel group of antibiotics, the glycylcyclines. This new derivative, like the first glycylcyclines, the *N,N*-dimethylglycylamido derivative of minocycline and 6-demethyl-6-deoxytetracycline, possesses activity against bacterial isolates containing the two major determinants responsible for tetracycline resistance: ribosomal protection and active efflux. The in vitro activities of TBG-MINO and the comparative agents were evaluated against strains with characterized tetracycline resistance as well as a spectrum of recent clinical aerobic and anaerobic gram-positive and gram-negative bacteria. TBG-MINO, with an MIC range of 0.25 to 0.5 µg/ml, showed good activity against strains expressing *tet*(M) (ribosomal protection), *tet*(A), *tet*(B), *tet*(C), *tet*(D), and *tet*(K) (efflux resistance determinants). TBG-MINO exhibited similar activity against methicillin-resistant *Staphylococcus aureus* (MRSA), penicillin-resistant streptococci, and vancomycin-resistant enterococci (MICs at which 90% of strains are inhibited, ≤0.5 µg/ml). TBG-MINO exhibited activity against a wide diversity of gram-negative aerobic and anaerobic bacteria, most of which were less susceptible to tetracycline and minocycline. The in vivo protective effects of TBG-MINO were examined against acute lethal infections in mice caused by *Escherichia coli*, *S. aureus*, and *Streptococcus pneumoniae* isolates. TBG-MINO, administered intravenously, demonstrated efficacy against infections caused by *S. aureus* including MRSA strains and strains containing *tet*(K) or *tet*(M) resistance determinants (median effective doses [ED₅₀s], 0.79 to 2.3 mg/kg of body weight). TBG-MINO demonstrated efficacy against infections caused by tetracycline-sensitive *E. coli* strains as well as *E. coli* strains containing either *tet*(M) or the efflux determinant *tet*(A), *tet*(B), or *tet*(C) (ED₅₀s, 1.5 to 3.5 mg/kg). Overall, TBG-MINO shows antibacterial activity against a wide spectrum of gram-positive and gram-negative aerobic and anaerobic bacteria including strains resistant to other chemotherapeutic agents. The in vivo protective effects, especially against infections caused by resistant bacteria, corresponded with the in vitro activity of TBG-MINO.

Tetracycline antibiotics were first isolated at Lederle Laboratories in 1945 and represented a significant advancement in the treatment of many infections (4, 7). However, due to an increased incidence of resistance among many bacteria (27), the use of the tetracyclines has been relegated to second- and third-line drug categories for most clinical indications (16, 25). The synthesis of new derivatives containing the *N,N*-dimethylglycylamido (DMG) substitution at the 9 position of minocycline and of 6-demethyl-6-deoxytetracycline (DMDOT) represented a significant advance in the tetracycline class of antibiotics (29). These new derivatives were named the glycylcyclines and were shown to be active against a wide spectrum of gram-positive and gram-negative bacteria, including resistant strains (5, 9, 12, 22, 31, 33, 34).

Derivatives in the minocycline series were found to be better tolerated than the DMDOT series in studies with rats (data not shown). In the present study we investigated the in vitro activity and in vivo efficacy of a new member of the glycylcyclines, TBG-MINO, the 9-*t*-butylglycylamido derivative of minocycline (Fig. 1), which was selected on the basis of its better tolerability and improved activity against tetracycline-resistant strains compared with those of DMG-DMDOT. The activity of TBG-MINO was determined against strains harboring characterized tetracycline resistance determinants and recent clinical isolates. The activities were compared with those of DMG-DMDOT, DMG-MINO, minocycline, tetracycline, and other

antimicrobial agents. The efficacy of TBG-MINO was compared with those of DMG-DMDOT and minocycline against murine systemic infections caused by bacterial strains harboring characterized tetracycline resistance determinants, laboratory strains, and recent clinical isolates adapted for murine infection.

MATERIALS AND METHODS

Organisms. Routine clinical isolates were collected from various medical centers in the United States and Canada between 1989 and 1994. Identification of each culture was done by conventional methods, as follows: gram-negative rods with the API 20E system (Analytab Products, Plainville, N.Y.) and the NF system (Remel, Lenexa, Kans.), anaerobes by the procedure outlined in the *Wadsworth Anaerobic Bacteriology Manual* (30), enterococci by biochemical tests as recommended by Facklam and Collins (6), streptococci with the API 20 Strep system (Analytab Products), and staphylococci with the Staph Trac system (Analytab Products). *Staphylococcus aureus* was also confirmed by a coagulase test. Methicillin resistance in *S. aureus* was determined with a plate containing oxacillin at 6 µg/ml, as described in the *Manual of Clinical Microbiology* (28). Penicillin-resistant (MICs, ≥2 µg/ml) *Streptococcus pneumoniae* isolates were obtained from A. Barry, Clinical Microbiology Institute, Tualatin, Ore., and S. Block, Bardstown, Ky. Strains with tetracycline resistance determinants and the vancomycin-resistant enterococci were obtained from the sources described previously (31). All isolates were stored frozen in skim milk at -70°C.

Antibiotics. Standard powders of TBG-MINO, DMG-MINO, DMG-DMDOT, vancomycin, minocycline, and tetracycline were obtained from Wyeth-Ayerst Laboratories, Pearl River, N.Y.; erythromycin was obtained from Sigma Chemical Co., St. Louis, Mo.; ciprofloxacin was obtained from Bayer Laboratories, West Haven, Conn.; ceftazidime was obtained from Glaxo Group Research, Ware, Herts, United Kingdom; and imipenem was obtained from Merck & Co., West Point, Pa.

In vitro susceptibility testing. The activities of the antibiotics were determined by the agar dilution method by following the recommendations of the National Committee for Clinical Laboratory Standards (20, 21). Mueller-Hinton II agar was used to test nonfastidious aerobic bacteria. The medium was supplemented with 5% sheep blood for the testing of streptococcal isolates and 15 µg of β-NAD per ml, 15 µg of hematin per ml, and 5 mg of yeast extract per ml for the testing

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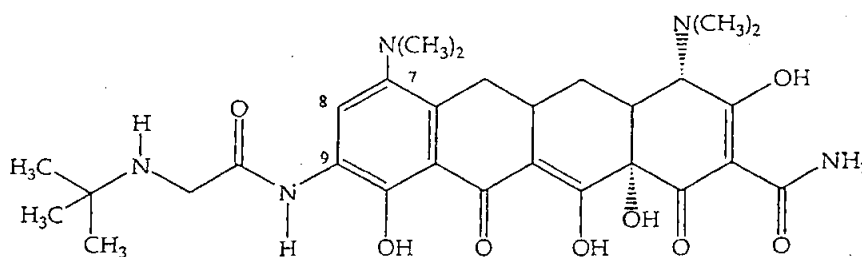


FIG. 1. Chemical structure of TBG-MINO.

of *Haemophilus influenzae* and *Moraxella catarrhalis*. GC agar supplemented with 1% hemoglobin and 1% IsoVital X was used to test *Neisseria gonorrhoeae*. Anaerobic bacteria were tested on Wilkins Chalgren agar supplemented with 5% lysed sheep blood and 0.001% vitamin K. The inocula, which were adjusted to the recommended densities (10^7 CFU/ml for aerobes and 10^8 CFU/ml for anaerobes), were applied to the surfaces of the agar plates with a Steers replicator. Test plates were incubated at 35°C for 18 to 24 h in ambient air for nonfastidious aerobic bacteria and streptococci and in CO_2 for *N. gonorrhoeae*, *H. influenzae*, and *M. catarrhalis*. Anaerobic bacteria were incubated in an anaerobic chamber (Coy Laboratories, Ann Arbor, Mich.) at 35°C for 48 h. The MIC was defined as the lowest concentration of the antimicrobial agent that completely inhibited the growth of the organism as detected by the unaided eye.

In vivo efficacy against murine infections. The therapeutic effects of the antibiotics were determined against acute lethal infections in mice (3) caused by minocycline-susceptible and minocycline-resistant gram-positive and gram-negative bacteria. Female CD-1 mice from Charles River Laboratories (weight, 20 ± 2 g each) were challenged by intraperitoneal injection of 0.5 ml of a bacterial suspension in either 5% hog gastric mucin or broth (10 to 100 50% lethal doses). Five to six doses of the antibiotic in phosphate-buffered saline (0.01 M; pH 7.4) were administered intravenously (0.2 ml) or orally (0.5 ml) at 0.5 h postinfection. For mice infected with *Escherichia coli* J3272 *Tc*^r *tet*(B), a second dose of the antibiotic was given 3 h later. In each test, five animals were treated with each dose. All the untreated controls died within 48 h of infection. The median effective dose (ED_{50}) was determined by probit analysis of the 7-day survival ratios pooled from three separate tests (8).

RESULTS

In vitro activity against tetracycline-resistant strains. The in vitro activity of TBG-MINO against prototype strains possessing characterized tetracycline resistance mechanisms is sum-

marized in Table 1. TBG-MINO had similar activity (MICs, ≤ 0.5 $\mu\text{g/ml}$) against tetracycline-susceptible and tetracycline-resistant *E. coli* strains carrying the efflux resistance determinants *tet*(A), *tet*(B), *tet*(C), and *tet*(D) and the strain carrying the ribosomal protection resistance determinant *tet*(M). TBG-MINO had activity similar to those of DMG-MINO and DMG-DMDOT against *E. coli* strains containing the *tet*(B) and *tet*(D) efflux resistance determinant and the ribosomal protection resistance determinant *tet*(M); however, TBG-MINO was more active than DMG-MINO and DMG-DMDOT against *E. coli* strains containing efflux resistance determinants *tet*(A) and *tet*(C). Minocycline demonstrated poorer activity (MIC range, 4 to >32 $\mu\text{g/ml}$) against all of the *E. coli* strains carrying the resistance determinants. TBG-MINO, with MICs of ≤ 0.5 $\mu\text{g/ml}$, was as active as DMG derivatives against the *tet*(K) (efflux)- and *tet*(M)-containing *S. aureus* strains. Minocycline was slightly more active than the glycyclines against *tet*(K)-containing *S. aureus* but had poorer activity against the three *S. aureus* strains containing *tet*(M).

In vitro activity against recent clinical isolates. TBG-MINO showed good activity against isolates of methicillin-resistant *S. aureus* (MRSA) and methicillin-resistant coagulase-negative staphylococci (MICs at which 90% of isolates are inhibited [MIC_{90}], ≤ 1 $\mu\text{g/ml}$). This activity was similar to that of minocycline and was 2 to 3 dilutions lower than those of DMG-MINO and DMG-DMDOT (Table 2). Against methicillin-

TABLE 1. In vitro activities of TBG-MINO, DMG-MINO, DMG-DMDOT, minocycline, and tetracycline against strains with characterized tetracycline resistance determinants

Organism	Strain	Resistance determinant	MIC ($\mu\text{g/ml}$)				
			TBG-MINO	DMG-MINO	DMG-DMDOT	Minocycline	Tetracycline
<i>E. coli</i>	UBMS 88-1	<i>tet</i> (B)	0.5	0.5	0.5	16	>32
<i>E. coli</i>	MC4100	<i>tet</i> (B)	0.5	0.5	0.5	8	>32
<i>E. coli</i>	J3272, pRP1	<i>tet</i> (A)	0.5	2	2	4	32
<i>E. coli</i>	J3272, pBR322	<i>tet</i> (C)	0.25	2	2	4	>32
<i>E. coli</i>	J3272, pRA1	<i>tet</i> (D)	0.25	0.25	0.25	8	>32
<i>E. coli</i>	UBMS 90-4	<i>tet</i> (M)	0.25	0.25	0.25	>32	>32
<i>E. coli</i>	UBMS 90-5	Sensitive	0.25	0.5	0.25	1	1
<i>E. coli</i>	ATCC 25922	Control	0.25	0.25	0.25	0.5	1
<i>S. aureus</i>	UBMS 88-7	<i>tet</i> (K)	0.5	1	1	0.25	>32
<i>S. aureus</i>	UBMS 88-5	<i>tet</i> (M)	0.5	0.25	0.25	4	>32
<i>S. aureus</i>	UBMS 90-1	<i>tet</i> (M)	0.25	0.25	0.12	4	>32
<i>S. aureus</i>	UBMS 90-2	<i>tet</i> (M)	0.25	0.25	0.25	2	32
<i>S. aureus</i>	UBMS 90-3	Sensitive	0.25	0.25	0.12	0.06	0.12
<i>S. aureus</i>	ATCC 29213	Control	0.5	0.25	0.25	0.06	0.25
<i>S. aureus</i>	Smith	Sensitive	0.25	0.25	0.12	0.06	0.12
<i>E. faecalis</i>	UBMS 90-6	<i>tet</i> (M)	0.25	0.12	0.25	16	>32
<i>E. faecalis</i>	ATCC 29212	Control	0.25	0.12	0.12	1	8
<i>N. gonorrhoeae</i>	6418	<i>tet</i> (M)	1	1	1	16	>32

TABLE 2. In vitro activities of TBG-MINO and comparative antibiotics against gram-positive isolates

Organism (no. of isolates)	Antibiotic	MIC ($\mu\text{g/ml}$)		
		Range	50%	90%
<i>Staphylococcus aureus</i> , methicillin resistant (12)	TBG-MINO	0.25-1	0.5	0.5
	DMG-MINO	0.12-2	0.25	2
	DMG-DMDOT	0.25-2	0.5	2
	Minocycline	0.03-4	0.12	1
	Tetracycline	0.25->32	0.5	>32
	Ciprofloxacin	0.25->32	8	32
	Vancomycin	0.5-2	1	1
<i>Staphylococcus aureus</i> , methicillin susceptible (13)	TBG-MINO	0.5	0.5	0.5
	DMG-MINO	0.25-0.5	0.5	0.5
	DMG-DMDOT	0.5	0.5	0.5
	Minocycline	0.12	0.12	0.12
	Tetracycline	0.5	0.5	0.5
	Ciprofloxacin	0.5-1	0.5	1
	Vancomycin	0.5-1	1	1
Coagulase-negative staphylococci, methicillin resistant (13)	TBG-MINO	0.25-2	0.5	1
	DMG-MINO	0.25-8	0.5	4
	DMG-DMDOT	0.12-8	1	8
	Minocycline	0.12-1	0.5	1
	Tetracycline	0.25->32	4	>32
	Ciprofloxacin	0.25->32	0.5	32
	Vancomycin	1-2	2	2
Coagulase-negative staphylococci, methicillin susceptible (16)	TBG-MINO	0.12-0.5	0.25	0.5
	DMG-MINO	0.12-0.5	0.25	0.5
	DMG-DMDOT	0.12-1	0.25	0.5
	Minocycline	0.06-0.5	0.12	0.25
	Tetracycline	0.12-32	0.5	32
	Ciprofloxacin	0.12-1	0.25	0.5
	Vancomycin	1-2	1	2
<i>Enterococcus faecalis</i> (11)	TBG-MINO	0.12-0.5	0.25	0.5
	DMG-MINO	0.06-0.25	0.25	0.25
	DMG-DMDOT	0.12-0.5	0.25	0.25
	Minocycline	0.06-16	8	8
	Tetracycline	0.25-32	32	32
	Ciprofloxacin	1-32	1	2
	Vancomycin	1-4	2	2
<i>Enterococcus faecium</i> (11)	TBG-MINO	0.12-0.25	0.25	0.25
	DMG-MINO	0.06-0.12	0.12	0.12
	DMG-DMDOT	0.12-0.25	0.12	0.12
	Minocycline	0.06-16	0.06	4
	Tetracycline	0.25->32	0.25	32
	Ciprofloxacin	1-4	4	4
	Vancomycin	0.25-2	1	2
<i>Enterococcus</i> spp., vancomycin resistant (10)	TBG-MINO	0.12-0.25	0.25	0.25
	DMG-MINO	0.06-0.25	0.12	0.25
	DMG-DMDOT	0.12-0.25	0.12	0.25
	Minocycline	0.06-8	0.06	8
	Tetracycline	0.25->32	0.25	>32
	Ciprofloxacin	0.5-4	4	4
	Vancomycin	>32	>32	>32
<i>Streptococcus pneumoniae</i> , penicillin resistant (10)	TBG-MINO	0.06-0.25	0.12	0.12
	DMG-MINO	0.03-0.12	0.06	0.06
	DMG-DMDOT	0.06-0.12	0.12	0.12
	Minocycline	0.12-8	4	4
	Tetracycline	0.5-32	32	32
	Vancomycin	0.25-0.5	0.5	0.5
<i>Streptococcus pneumoniae</i> , penicillin susceptible (10)	TBG-MINO	0.06-0.12	0.06	0.12
	DMG-MINO	0.06-0.25	0.06	0.12
	DMG-DMDOT	0.06-0.5	0.06	0.12
	Minocycline	0.06-0.5	0.12	0.12
	Tetracycline	0.12-4	0.25	0.5
	Vancomycin	0.12-1	0.5	0.5
<i>Streptococcus pyogenes</i> (10)	TBG-MINO	0.12-0.5	0.12	0.25
	DMG-MINO	0.06-0.12	0.12	0.12
	DMG-DMDOT	0.12	0.12	0.12
	Minocycline	0.06-0.25	0.06	0.12
	Tetracycline	0.25-16	0.25	0.25
	Vancomycin	0.5	0.5	0.5
<i>Streptococcus agalactiae</i> (10)	TBG-MINO	0.12-0.5	0.12	0.25
	DMG-MINO	0.12-0.5	0.12	0.5
	DMG-DMDOT	0.12-1	0.12	0.25
	Minocycline	0.12-16	16	16
	Tetracycline	0.25-32	32	32
	Vancomycin	0.5-1	0.5	0.5

susceptible staphylococci, the three glycylicycline derivatives had equivalent activities (MICs, $\leq 0.5 \mu\text{g/ml}$). TBG-MINO and the DMG derivatives demonstrated activity against *Enterococcus faecalis* and *Enterococcus faecium*, including vancomycin-resistant strains (MIC₉₀s, $\leq 0.5 \mu\text{g/ml}$). The three glycylicyclines, minocycline, and tetracycline exhibited good activity against *Streptococcus pyogenes* and penicillin-susceptible *S. pneumoniae*; however, TBG-MINO and the DMG derivatives were 32 to 64 times more active than minocycline against *Streptococcus agalactiae* and penicillin-resistant *S. pneumoniae*. No differences in the activity of TBG-MINO between penicillin-susceptible and penicillin-resistant *S. pneumoniae* isolates were noted. In general, TBG-MINO, with MICs of $\leq 1 \mu\text{g/ml}$, displayed greater activity than the other comparative antibiotics, vancomycin, ciprofloxacin, and erythromycin, against most of the staphylococcal and enterococcal isolates tested.

TBG-MINO, with a range of MICs of 0.5 to 8 $\mu\text{g/ml}$, was 4 to 32 times more active than minocycline against clinical isolates of *E. coli*, *Shigella* spp., *Citrobacter diversus*, *Salmonella* spp., *Providencia* spp., *Morganella morganii*, and *N. gonorrhoeae* (Table 3). TBG-MINO was generally as active or more active than minocycline against most strains of *Klebsiella* spp., *Citrobacter freundii*, *Enterobacter* spp., *Serratia marcescens*, *Proteus mirabilis*, *Proteus vulgaris*, *Burkholderia cepacia*, and *Pseudomo-*

nas aeruginosa. In general, the three glycylicyclines demonstrated similar activities against gram-negative isolates; however, greater activity was observed with TBG-MINO than with DMG-MINO or DMG-DMDOT (MIC₉₀s, ≤ 0.5 versus 4 $\mu\text{g/ml}$, respectively) against *E. coli* strains for which minocycline MICs were elevated (MIC₉₀, 16 $\mu\text{g/ml}$). TBG-MINO, DMG-MINO, and DMG-DMDOT were generally less active than ciprofloxacin, imipenem, and ceftazidime against most gram-negative bacteria. However, organisms resistant to these antibiotics showed no cross-resistance with the glycylicyclines.

TBG-MINO and the other glycylicycline derivatives, with a range of MICs of 0.12 to 2 $\mu\text{g/ml}$, were more active than minocycline against *Bacteroides* spp., *Prevotella* spp., *Clostridium difficile*, and anaerobic gram-positive cocci (Table 4). For some members of the *Bacteroides fragilis* group, the MICs of TBG-MINO but not those of DMG-MINO or DMG-DMDOT were found to be elevated (1 to 2 $\mu\text{g/ml}$). In general, the three glycylicyclines were more active than cefoxitin but were less active than imipenem against most of the anaerobic bacteria tested.

In vivo efficacy. Administered as a single intravenous dose, TBG-MINO showed efficacy against infections caused by tetracycline-susceptible and tetracycline-resistant *S. aureus* and *E. coli* strains in mice (Table 5 and 6). Against an infection with

TABLE 3. In vitro activities of TBG-MINO and comparative antibiotics against gram-negative isolates

Organism (no. of isolates)	Antibiotic	MIC ($\mu\text{g/ml}$)			Organism (no. of isolates)	Antibiotic	MIC ($\mu\text{g/ml}$)		
		Range	50%	90%			Range	50%	90%
<i>Escherichia coli</i> (minocycline MIC, $\approx 1 \mu\text{g/ml}$) (32)	TBG-MINO	0.25-1	0.5	0.5	<i>Enterobacter cloacae</i> (10)	TBG-MINO	1-2	1	2
	DMG-MINO	0.25-4	0.5	4		DMG-MINO	1-2	1	2
	DMG-DMDOT	0.25-4	1	4		DMG-DMDOT	1-2	1	2
	Minocycline	1-32	8	16		Minocycline	2-4	4	4
	Tetracycline	2->32	>32	>32		Tetracycline	2-4	4	4
	Ciprofloxacin	0.008-32	0.008	0.015		Ciprofloxacin	≤ 0.004 -0.06	0.03	0.03
	Imipenem	0.06-0.25	0.12	0.12		Imipenem	0.25	0.25	0.25
<i>Escherichia coli</i> (minocycline MIC, $\leq 0.5 \mu\text{g/ml}$) (14)	Ceftazidime	0.06-1	0.12	0.25		Ceftazidime	0.12->32	0.25	>32
	TBG-MINO	0.25-0.5	0.5	0.5	<i>Enterobacter aerogenes</i> (10)	TBG-MINO	1	1	1
	DMG-MINO	0.25-0.5	0.25	0.5		DMG-MINO	1	1	1
	DMG-DMDOT	0.5	0.5	0.5		DMG-DMDOT	1	1	1
	Minocycline	0.25-0.5	0.5	0.5		Minocycline	2	2	2
	Tetracycline	1-2	1	2		Tetracycline	2	2	2
	Ciprofloxacin	≤ 0.004 -0.25	≤ 0.004	0.03		Ciprofloxacin	≤ 0.004 -0.03	0.015	0.03
	Imipenem	0.06-0.12	0.12	0.12		Imipenem	0.25-2	0.25	2
<i>Shigella</i> spp. (26)	Ceftazidime	0.06-0.25	0.12	0.12		Ceftazidime	0.12->32	0.12	>32
	TBG-MINO	0.12-0.5	0.25	0.5	<i>Providencia</i> spp. (10)	TBG-MINO	4-8	4	8
	DMG-MINO	0.12-1	0.25	0.5		DMG-MINO	2-8	8	8
	DMG-DMDOT	0.12-1	0.5	0.5		DMG-DMDOT	4-8	4	8
	Minocycline	0.25-16	2	4		Minocycline	4->32	16	>32
	Tetracycline	1->32	>32	>32		Tetracycline	4->32	>32	>32
	Ciprofloxacin	≤ 0.004 -0.015	≤ 0.004	0.008		Ciprofloxacin	≤ 0.004 -0.25	0.03	0.25
	Imipenem	0.06-0.5	0.12	0.25		Imipenem	0.25-2	1	2
<i>Klebsiella pneumoniae</i> (10)	Ceftazidime	0.06-0.12	0.12	0.12		Ceftazidime	0.03-4	0.06	4
	TBG-MINO	0.5-2	1	2	<i>Proteus mirabilis</i> (15)	TBG-MINO	2-8	4	8
	DMG-MINO	0.5-1	1	1		DMG-MINO	1-16	4	8
	DMG-DMDOT	0.5-1	1	1		DMG-DMDOT	0.12-2	1	1
	Minocycline	1-4	2	4		Minocycline	2-32	8	16
	Tetracycline	1-4	2	2		Tetracycline	1-32	16	32
	Ciprofloxacin	0.008-0.03	0.03	0.03		Ciprofloxacin	0.008-0.06	0.06	0.06
	Imipenem	0.12-0.5	0.12	0.25		Imipenem	0.003-0.12	0.06	0.12
<i>Klebsiella oxytoca</i> (10)	Ceftazidime	0.06-0.5	0.12	0.12		Ceftazidime	0.015-0.06	0.03	0.03
	TBG-MINO	1	1	1	<i>Proteus vulgaris</i> (15)	TBG-MINO	1-4	4	4
	DMG-MINO	1	1	1		DMG-MINO	0.5-4	1	2
	DMG-DMDOT	1	1	1		DMG-DMDOT	0.25-1	0.5	1
	Minocycline	2-8	2	2		Minocycline	0.5-8	2	4
	Tetracycline	2->32	2	2		Tetracycline	0.5->32	8	32
	Ciprofloxacin	0.008-0.03	0.015	0.015		Ciprofloxacin	0.008-0.25	0.015	0.12
	Imipenem	0.12-0.25	0.12	0.25		Imipenem	0.03-0.12	0.06	0.12
<i>Citrobacter freundii</i> (10)	Ceftazidime	0.06-0.5	0.12	0.12		Ceftazidime	0.015-0.25	0.03	0.06
	TBG-MINO	0.5-8	1	2	<i>Morganella morganii</i> (10)	TBG-MINO	2-8	4	4
	DMG-MINO	0.5-8	1	2		DMG-MINO	1-4	4	4
	DMG-DMDOT	1-8	1	1		DMG-DMDOT	1-4	2	2
	Minocycline	1-32	4	4		Minocycline	2->32	4	16
	Tetracycline	1-16	2	2		Tetracycline	2->32	2	>32
	Ciprofloxacin	≤ 0.004 -16	0.015	0.12		Ciprofloxacin	≤ 0.004 -1	0.008	0.03
	Imipenem	0.25-2	0.5	1		Imipenem	2-4	2	4
<i>Citrobacter diversus</i> (10)	Ceftazidime	0.12->32	0.5	8		Ceftazidime	0.06-32	0.12	32
	TBG-MINO	0.5-2	1	1	<i>Pseudomonas aeruginosa</i> (10)	TBG-MINO	8-16	16	16
	DMG-MINO	0.5-2	1	1		DMG-MINO	4-8	8	8
	DMG-DMDOT	1-2	1	1		DMG-DMDOT	4-16	8	8
	Minocycline	1-4	2	4		Minocycline	2-8	8	8
	Tetracycline	2-8	2	4		Tetracycline	8->32	16	>32
	Ciprofloxacin	≤ 0.004 -0.06	0.008	0.06		Ciprofloxacin	0.12-2	0.25	2
	Imipenem	0.06-12	0.06	0.12		Imipenem	0.5-2	1	1
<i>Salmonella</i> spp. (14)	Ceftazidime	0.12-0.5	0.12	0.5		Ceftazidime	0.5-32	2	16
	TBG-MINO	0.25-2	1	1	<i>Burkholderia cepacia</i> (10)	TBG-MINO	0.5-4	2	4
	DMG-MINO	0.5-4	0.5	1		DMG-MINO	0.5-4	1	4
	DMG-DMDOT	0.5-4	0.5	1		DMG-DMDOT	0.5-4	2	4
	Minocycline	0.5-32	2	16		Minocycline	0.06-2	0.5	2
	Tetracycline	1->32	2	>32		Tetracycline	1->32	2	4
	Ciprofloxacin	≤ 0.004 -0.03	0.015	0.03		Ciprofloxacin	0.03-4	0.12	2
	Imipenem	0.06-0.25	0.12	0.25		Imipenem	0.06-8	4	8
<i>Serratia marcescens</i> (10)	Ceftazidime	0.12-0.5	0.25	0.5		Ceftazidime	0.5-4	2	4
	TBG-MINO	4-8	4	4	<i>Stenotrophomonas maltophilia</i> (10)	TBG-MINO	1-4	2	4
	DMG-MINO	4-8	4	8		DMG-MINO	0.5-4	1	2
	DMG-DMDOT	4-8	4	4		DMG-DMDOT	2-8	4	8
	Minocycline	4-8	8	8		Minocycline	0.06-0.5	0.12	0.25
	Tetracycline	8->32	32	>32		Tetracycline	8-16	16	16
	Ciprofloxacin	0.008-2	0.12	0.25		Ciprofloxacin	1-4	2	4
	Imipenem	0.25-2	0.5	2		Imipenem	>32	>32	>32
	Ceftazidime	0.12-1	0.25	0.5		Ceftazidime	4->32	8	>32

Continued on following page

TABLE 3—Continued

Organism (no. of isolates)	Antibiotic	MIC (μ g/ml)		
		Range	50%	90%
<i>Moraxella catarrhalis</i> (14)	TBG-MINO	0.12–0.25	0.12	0.25
	DMG-MINO	0.06–0.12	0.12	0.12
	DMG-DMDOT	0.12–0.25	0.12	0.25
	Minocycline	0.008–0.06	0.03	0.06
	Tetracycline	0.06–0.25	0.12	0.25
	Ciprofloxacin	0.03–0.06	0.03	0.06
	Imipenem	0.008–0.06	0.015	0.06
<i>Neisseria gonorrhoeae</i> (22)	Ceftazidime	0.015–0.12	0.015	0.06
	TBG-MINO	0.25–1	0.5	1
	DMG-MINO	0.12–1	0.25	0.5
	DMG-DMDOT	0.25–1	0.5	1
	Minocycline	0.25–>32	0.5	32
	Tetracycline	0.5–>32	1	>32
	Ciprofloxacin	≤ 0.004	≤ 0.004	≤ 0.004
<i>Haemophilus influenzae</i> (15)	Imipenem	0.015–0.12	0.06	0.12
	Ceftazidime	0.015–0.25	0.03	0.06
	TBG-MINO	0.25–1	0.5	1
	DMG-MINO	0.25–0.5	0.25	0.5
	DMG-DMDOT	0.25–0.5	0.5	0.5
	Minocycline	0.12–0.25	0.12	0.25
	Tetracycline	0.12–8	0.25	0.5
	Ciprofloxacin	≤ 0.004 –0.03	0.015	0.03
	Imipenem	1–8	2	4
	Ceftazidime	0.015–0.25	0.12	0.12

S. aureus Smith, a tetracycline-susceptible strain, all three compounds, TBG-MINO, DMG-DMDOT, and minocycline, displayed efficacy (ED_{50} s, 0.64, 0.51, and 0.53 mg/kg of body weight, respectively) when they were administered intravenously; however, when they were administered orally, TBG-MINO and DMG-DMDOT were 40- to 60-fold less efficacious (Table 5). In contrast, when administered orally minocycline exhibited efficacy equivalent to that achieved when it was administered intravenously against *S. aureus* Smith infection (ED_{50} , 0.52 mg/kg). Due to the poor efficacy in mice noted when the drugs were given by the oral route, other in vivo tests were performed with only intravenous administration. TBG-MINO and DMG-DMDOT were moderately more efficacious than minocycline against an infection with *S. aureus* UBMS 90-2 [a *tet*(M) (ribosomal protection)-containing strain] (Table 6). TBG-MINO, DMG-DMDOT, and minocycline had comparable efficacies against an infection caused by *S. aureus* UBMS 88-7, a *tet*(K) efflux resistance determinant-containing strain (ED_{50} s, 2.1, 3.1, and 2.0 mg/kg, respectively). TBG-MINO and DMG-DMDOT showed protective efficacy against an infection caused by *S. aureus* NEMC 89-4 (a tetracycline-susceptible, methicillin-resistant strain), but minocycline was slightly more effective. Against infections caused by an MRSA strain containing the *tet*(M) resistance determinant (strain ID 4729) and an MRSA strain carrying both *tet*(M) and *tet*(K) resistance determinants (strain ID 2371), TBG-MINO and DMG-DMDOT showed efficacies which exceeded that of minocycline by approximately two and five times, respectively. Comparable efficacies against infections caused by *S. pneumoniae* were obtained with TBG-MINO and DMG-DMDOT, regardless of the strain's susceptibility to penicillin (range of ED_{50} s, 0.53 to 1.9 mg/kg). Minocycline was slightly less effective against infections caused by penicillin-susceptible *S. pneumoniae* and was >30 times less effective than the glycolcyclines against a penicillin-resistant *S. pneumoniae* infection (ED_{50} , 20 mg/kg).

TBG-MINO, DMG-DMDOT, and minocycline were observed to have similar efficacies against an infection caused by the tetracycline-susceptible strain *E. coli* 311, with ED_{50} s of 1.7, 1.5, and 3.2 mg/kg, respectively. Against infections caused by *E. coli* strains containing *tet*(A) or *tet*(C) efflux resistance

TABLE 4. In vitro activities of TBG-MINO and comparative antibiotics against anaerobic bacteria

Organism (no. of isolates)	Antibiotic	MIC (μ g/ml)		
		Range	50%	90%
<i>Bacteroides fragilis</i> group (12)	TBG-MINO	0.25–2	0.5	2
	DMG-MINO	0.25–0.5	0.25	0.5
	DMG-DMDOT	0.25–0.5	0.25	0.5
	Minocycline	0.06–4	2	4
	Cefoxitin	2–>32	16	>32
	Imipenem	≤ 0.06 –2	0.12	2
<i>Bacteroides fragilis</i> (14)	TBG-MINO	0.5–8	2	2
	DMG-MINO	0.25–2	1	1
	DMG-DMDOT	0.5–2	1	2
	Minocycline	≤ 0.06 –8	8	8
	Cefoxitin	1–8	8	8
	Imipenem	≤ 0.06 –0.25	≤ 0.06	0.12
<i>Prevotella</i> spp. (11)	TBG-MINO	0.12–1	0.5	1
	DMG-MINO	≤ 0.06 –0.5	0.25	0.5
	DMG-DMDOT	≤ 0.06 –2	0.5	2
	Minocycline	≤ 0.06 –16	8	16
	Cefoxitin	0.25–4	1	2
	Imipenem	≤ 0.06	≤ 0.06	≤ 0.06
<i>Clostridium difficile</i> (10)	TBG-MINO	≤ 0.06 –0.25	0.12	0.12
	DMG-MINO	≤ 0.06 –0.12	0.12	0.12
	DMG-DMDOT	≤ 0.06 –0.12	0.12	0.12
	Minocycline	≤ 0.06 –4	0.03	4
	Cefoxitin	>32	>32	>32
	Imipenem	2–16	4	4
<i>Clostridium perfringens</i> (10)	TBG-MINO	0.12–4	0.5	1
	DMG-MINO	0.12–4	0.25	2
	DMG-DMDOT	0.12–4	0.25	2
	Minocycline	≤ 0.06 –8	≤ 0.06	4
	Cefoxitin	0.25–1	0.5	1
	Imipenem	≤ 0.06	≤ 0.06	≤ 0.06
Anaerobic gram-positive cocci (15)	TBG-MINO	≤ 0.06 –0.25	0.12	0.25
	DMG-MINO	≤ 0.06 –0.12	≤ 0.06	0.12
	DMG-DMDOT	≤ 0.06 –0.5	0.12	0.5
	Minocycline	0.12–16	4	16
	Cefoxitin	≤ 0.06 –16	0.12	16
	Imipenem	≤ 0.06 –1	≤ 0.06	1

determinants, TBG-MINO (ED_{50} s, 1.6 and 1.5 mg/kg, respectively) exhibited efficacy that was approximately three times that of DMG-DMDOT and more than nine times that of minocycline. Against an infection caused by *E. coli* UBMS 90-4, a laboratory strain in which the *tet*(M) resistance determinant mechanism was inserted, both TBG-MINO and DMG-DMDOT, with ED_{50} s of 3.5 and 2.1 mg/kg, respectively, demonstrated good efficacy, while minocycline was not therapeutically effective at doses of up to 32 mg/kg. Intravenous administration of TBG-MINO or DMG-DMDOT resulted in good

TABLE 5. In vivo activities of TBG-MINO, DMG-DMDOT, and minocycline against experimental acute lethal *S. aureus* Smith infection in mice^a

Antibiotic	Route	ED_{50} (mg/kg) (95% confidence limit)	MIC (μ g/ml)
TBG-MINO	Intravenous	0.64 (0.51–0.80)	0.25
TBG-MINO	Oral	36 (28–45)	0.25
DMG-DMDOT	Intravenous	0.51 (0.41–0.64)	0.12
DMG-DMDOT	Oral	21 (16–26)	0.12
Minocycline	Intravenous	0.53 (0.40–0.70)	0.06
Minocycline	Oral	0.52 (0.40–0.69)	0.06

^a Challenge dose, 6.2×10^5 CFU/mouse.

TABLE 6. In vivo activities of TBG-MINO, DMG-DMDOT, and minocycline against experimental acute lethal infections in mice

Infection (resistance determinant or resistance; challenge dose [CFU/mouse])	Intravenous treatment	ED ₅₀ (mg/kg) (95% confidence limit)	MIC (μg/ml)
<i>Staphylococcus aureus</i> UBMS 90-2 (<i>tet</i> (M), ribosomal protection; 7.9×10^7)	TBG-MINO	1.0 (0.87–1.3)	0.12
	DMG-DMDOT	0.68 (0.56–0.81)	0.12
	Minocycline	1.8 (1.5–2.2)	2.0
<i>Staphylococcus aureus</i> UBMS 88-7649(pUB111) (<i>tet</i> (K), efflux; 9.0×10^7)	TBG-MINO	2.1 (1.8–2.6)	0.5
	DMG-DMDOT	3.1 (2.5–3.7)	1.0
	Minocycline	2.0 (1.6–2.4)	0.25
<i>Staphylococcus aureus</i> NEMC 89-4 (MRSA; 5.3×10^7)	TBG-MINO	0.79 (0.64–0.97)	0.50
	DMG-DMDOT	0.48 (0.39–0.59)	0.25
	Minocycline	0.31 (0.25–0.38)	0.12
<i>Staphylococcus aureus</i> ID 4729 (MRSA, <i>tet</i> (M); 1.3×10^8)	TBG-MINO	0.84 (0.69–1.0)	0.5
	DMG-DMDOT	0.53 (0.43–0.64)	0.25
	Minocycline	1.6 (1.3–2.0)	4.0
<i>Staphylococcus aureus</i> ID 2371 (MRSA, <i>tet</i> (M), <i>tet</i> (K); 1.3×10^8)	TBG-MINO	2.3 (1.9–2.7)	1.0
	DMG-DMDOT	3.0 (2.4–3.6)	2.0
	Minocycline	16 (13–20)	4.0
<i>Streptococcus pneumoniae</i> ATCC 6301 (penicillin susceptible; 3.3×10^1)	TBG-MINO	1.3 (1.1–1.6)	0.12
	DMG-DMDOT	1.3 (1.1–1.6)	0.06
	Minocycline	3.9 (3.2–4.8)	0.12
<i>Streptococcus pneumoniae</i> ATCC 10015 (penicillin susceptible; 1.5×10^1)	TBG-MINO	1.7 (1.4–2.2)	0.12
	DMG-DMDOT	1.9 (1.5–2.4)	0.12
	Minocycline	3.5 (2.8–4.4)	0.12
<i>Streptococcus pneumoniae</i> GS 1894 (penicillin resistant; 3.7×10^1)	TBG-MINO	0.61 (0.48–0.77)	0.12
	DMG-DMDOT	0.53 (0.42–0.67)	0.12
	Minocycline	20 (16–26)	4
<i>Escherichia coli</i> 311 (susceptible; 2.3×10^6)	TBG-MINO	1.7 (1.4–2.1)	0.5
	DMG-DMDOT	1.5 (1.2–1.8)	0.5
	Minocycline	3.2 (2.6–4.0)	1.0
<i>Escherichia coli</i> J3272 (pRP1) (<i>tet</i> (A), efflux; 1.6×10^7)	TBG-MINO	1.6 (1.0–2.6)	0.5
	DMG-DMDOT	4.6 (2.9–7.5)	2.0
	Minocycline	16.0 (9.8–26.0)	4.0
<i>Escherichia coli</i> J3272(pBR322) (<i>tet</i> (C), efflux; 2.6×10^7)	TBG-MINO	1.5 (1.3–1.9)	0.25
	DMG-DMDOT	5.0 (4.1–6.4)	2.0
	Minocycline	14.0 (11.0–17.0)	4.0
<i>Escherichia coli</i> UBMS 90-4 (<i>tet</i> (M), ribosomal protection; 6.6×10^7)	TBG-MINO	3.5 (2.8–4.3)	0.25
	DMG-DMDOT	2.1 (1.8–2.6)	0.25
	Minocycline	>32.0	>32.0
<i>Escherichia coli</i> UBMS 88-1, J3272TcR (<i>tet</i> (B), efflux; 3.9×10^7)	TBG-MINO	3.9 (3.2–4.9)	0.5
	DMG-DMDOT	3.1 (2.5–3.8)	0.5
	Minocycline	>32.0	32.0
<i>Escherichia coli</i> NEMC 87-30 (minocycline resistant; 5.3×10^7)	TBG-MINO	1.6 (1.3–1.9)	0.5
	DMG-DMDOT	2.0 (1.7–2.4)	0.5
	Minocycline	>32.0	32.0

efficacy against an infection caused by *E. coli* UBMS 88-1, a strain carrying the *tet*(B) efflux resistance determinant, while minocycline was not efficacious. Both TBG-MINO and DMG-DMDOT showed efficacy (ED₅₀s, ≤ 2.0 mg/kg) against an infection caused by a minocycline-resistant *E. coli* clinical isolate (NEMC 87-30).

DISCUSSION

Previous studies (5, 9, 12, 22, 31, 33, 34) demonstrated that the DMG modification of the 9 position of the tetracycline molecule (29), i.e., DMG-MINO and DMG-DMDOT, resulted in drugs that have the ability to overcome the two major mechanisms responsible for tetracycline resistance, i.e., ribosomal protection or active efflux of drug out of the bacterial cell (1, 2, 13–15, 24, 26, 27). TBG-MINO, the 9-*i*-butylglycylamido de-

rivative of minocycline, a recently synthesized member of the glycylcycline family of compounds, possesses a spectrum of activity similar to those DMG-MINO and DMG-DMDOT against most of the strains carrying the tetracycline resistance determinants. However, TBG-MINO has improved in vitro and in vivo activities against *E. coli* strains carrying the *tet*(A) or *tet*(C) resistance determinant.

The activity of TBG-MINO matched the activities of DMG-MINO and DMG-DMDOT against recent clinical gram-negative and -positive aerobic and anaerobic isolates, including minocycline- and tetracycline-resistant isolates. Differences in activities between TBG-MINO, DMG-MINO, and DMG-DMDOT were noted against some strains of *E. coli*, against which TBG-MINO was more active than DMG-MINO or DMG-DMDOT. Because TBG-MINO demonstrated better activity

when it was tested against prototype strains of *E. coli* with *tet*(A) or *tet*(C) resistance determinants, it is possible that some of these clinical isolates may contain one or both of these resistance determinants. The MIC₉₀s of TBG-MINO for MRSA and methicillin-resistant coagulase-negative staphylococci were also lower. The MICs of DMG-DMDOT and DMG-MINO were elevated for two of the clinical MRSA strains, which contained both *tet*(K) and *tet*(M) resistance determinants, but these strains were more sensitive to TBG-MINO (data not shown). Because all three glycylicyclines showed good activities against *tet*(M)-carrying strains, the slightly improved activity of TBG-MINO might reflect the slightly better inherent activity noted against *tet*(K)-containing strains. TBG-MINO and DMG-MINO were less active than DMG-DMDOT against *Proteus* spp. and *M. morganii*.

The improved in vitro activity of TBG-MINO was also observed in vivo when its activity against acute lethal infections in mice was tested. When it was dosed intravenously, TBG-MINO was as effective as minocycline against infections caused by minocycline-susceptible bacteria including MRSA and *tet*(K)-containing *S. aureus*. However, the ED₅₀s of TBG-MINO and DMG-DMDOT against infections caused by MRSA that also contained *tet*(M) were lower than those of minocycline. Infections caused by *E. coli* strains carrying *tet*(A), *tet*(B), *tet*(C), or *tet*(M) were more responsive to treatment with TBG-MINO or DMG-DMDOT than to treatment with minocycline. The activity of TBG-MINO, however, exceeded the activity of DMG-DMDOT against infections caused by the *tet*(A)- and *tet*(C)-containing strains, thus reflecting the improved in vitro activity of TBG-MINO over that of DMG-DMDOT. Both TBG-MINO and DMG-DMDOT had poor efficacies when they were administered orally.

The ability of TBG-MINO to overcome the major tetracycline resistance mechanisms and extend its spectrum of activity to include multidrug-resistant staphylococci, penicillin-resistant *S. pneumoniae*, vancomycin-resistant enterococci, anaerobes, and minocycline-resistant bacteria while retaining activity against minocycline-susceptible microorganisms makes it an attractive new antibacterial agent. Resistance among *S. pneumoniae*, *Enterococcus* spp., and MRSA is becoming an increasing medical problem worldwide (10, 11, 17, 18, 19, 23, 32), with reduced therapeutic options and an increased need for new antimicrobial agents. TBG-MINO at concentrations of ≤ 0.5 μ g/ml inhibited all strains of penicillin-resistant *S. pneumoniae*, vancomycin-resistant *Enterococcus* spp., and MRSA. Therefore, additional evaluation of TBG-MINO is warranted.

REFERENCES

- Chopra, I., P. M. Hawkey, and M. Hilton. 1992. Tetracyclines, molecular and clinical aspects. *J. Antimicrob. Chemother.* 29:245-277.
- Chopra, I., S. Shales, and P. Ball. 1982. Tetracycline resistant determinants from groups A to D vary in their ability to confer decreased accumulation of tetracycline derivatives by *Escherichia coli*. *J. Gen. Microbiol.* 128:689-692.
- Cleeland, R., and E. Squires. 1991. Evaluation of new antimicrobials in vitro and in experimental animal infections, p. 752-783. In V. Lorian (ed.), *Antibiotics in laboratory medicine*, 3rd ed. The Williams & Wilkins Co., Baltimore, Md.
- Duggar, B. M. 1948. Aureomycin: a product of the continuing search for new antibiotics. *Ann. N. Y. Acad. Sci.* 51:177-181.
- Eliopoulos, G., C. Wennersten, G. Cole, and R. Moellering. 1994. In vitro activities of two glycylicyclines against gram-positive bacteria. *Antimicrob. Agents Chemother.* 38:534-541.
- Facklam, R., and D. Collins. 1989. Identification of *Enterococcus* species isolated from human infections by a conventional test scheme. *J. Clin. Microbiol.* 27:731-734.
- Finland, M. 1974. Twenty-fifth anniversary of the discovery of aureomycin: the place of the tetracyclines in antimicrobial therapy. *Clin. Pharmacol. Ther.* 15:3-8.
- Finney, D. J. 1971. *Probit analysis*, 3rd ed. Cambridge University Press, London, United Kingdom.
- Goldstein, F. W., M. D. Kitzis, and J. F. Acar. 1994. *N,N*-Dimethylglycyl-amido derivative of minocycline and 6-demethyl-6-deoxytetracycline, two new glycylicyclines highly effective against tetracycline-resistant gram-positive cocci. *Antimicrob. Agents Chemother.* 38:2218-2220.
- Grayson, M., G. Eliopoulos, C. Wennersten, K. Ruoff, P. Girolami, M. Ferraro, and R. Moellering. 1991. Increasing resistance to β -lactam antibiotics among clinical isolates of *Enterococcus faecium*: a 22-year review at one institution. *Antimicrob. Agents Chemother.* 35:2180-2184.
- Handwerker, S., B. Raucher, D. Altarac, J. Monka, S. Marchione, K. Singh, B. Murray, J. Wolff, and B. Waters. 1993. Nosocomial outbreak due to *Enterococcus faecium* highly resistant to vancomycin, penicillin and gentamicin. *Clin. Infect. Dis.* 16:750-755.
- Kenny, G. E., and F. D. Cartright. 1994. Susceptibilities of *Mycoplasma hominis*, *Mycoplasma pneumoniae*, and *Ureaplasma urealyticum* to new glycylicyclines in comparison with those to older tetracyclines. *Antimicrob. Agents Chemother.* 38:2628-2632.
- Levy, S. B. 1984. Resistance to the tetracyclines, p. 191-240. In L. E. Bryan (ed.), *Antimicrobial drug resistance*. Academic Press, Inc., New York, N.Y.
- Levy, S. B. 1989. Evolution and spread of tetracycline resistance determinants. *J. Antimicrob. Chemother.* 24:1-3.
- Levy, S. B., L. M. McMurry, V. Burdett, P. Courvalin, W. Hillen, M. C. Roberts, and D. E. Taylor. 1989. Nomenclature for tetracycline resistance determinants. *Antimicrob. Agents Chemother.* 33:1373-1374.
- Mandell, G. L. 1993. *Principles and practice of infectious diseases*. Churchill Livingstone Inc., New York, N.Y.
- Mason, E., S. Kaplan, L. Lamberth, and J. Tillman. 1992. Increased rate of isolation of penicillin-resistant *Streptococcus pneumoniae* in a children's hospital and in vitro susceptibilities to antibiotics of potential therapeutic use. *Antimicrob. Agents Chemother.* 36:1703-1707.
- Mulligan, M., K. Murray-Leisure, B. Ribner, H. Standiford, J. John, J. Korvick, C. Kauffman, and V. Yu. 1993. Methicillin-resistant *Staphylococcus aureus*: a consensus review of the microbiology, pathogenesis and epidemiology with implications for prevention and management. *Am. J. Med.* 94:313-328.
- Murray, B. 1990. The life and times of the enterococci. *Clin. Microbiol. Rev.* 3:46-65.
- National Committee for Clinical Laboratory Standards. 1997. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically. Approved standard M7-A4, vol. 17, no. 2. National Committee for Clinical Laboratory Standards, Wayne, Pa.
- National Committee for Clinical Laboratory Standards. 1997. Methods for antimicrobial susceptibility testing of anaerobic bacteria. Approved standard M11-A4, vol. 17, no. 22. National Committee for Clinical Laboratory Standards, Wayne, Pa.
- Nord, C., A. Lindmark, and I. Persson. 1993. In vitro activity of DMG-Mino and DMG-Dmdot, two new glycylicyclines, against anaerobic bacteria. *Eur. J. Clin. Microbiol. Infect. Dis.* 12:784-786.
- Sahn, D., J. Kissinger, M. Gilmore, P. Murray, R. Mulder, J. Solliday, and B. Clarke. 1989. In vitro susceptibility studies of vancomycin-resistant *Enterococcus faecium*. *Antimicrob. Agents Chemother.* 33:1588-1591.
- Salysers, A. A., B. S. Spear, and N. G. Shoemaker. 1990. New perspectives on tetracycline resistance. *Mol. Microbiol.* 4:151-156.
- Sanford, J. 1997. *Guide to antimicrobial therapy*. Antimicrobial Therapy, Inc., West Bethesda, Md.
- Shales, S. W., I. Chopra, and P. R. Ball. 1980. Evidence of more than one mechanism of plasmid-determined tetracycline resistance in *Escherichia coli*. *J. Gen. Microbiol.* 121:221-229.
- Speer, B. S., N. B. Shoemaker, and A. A. Salysers. 1992. Bacterial resistance to tetracycline: mechanisms, transfer, and clinical significance. *Clin. Microbiol. Rev.* 5:387-399.
- Stratton, C. W., and R. C. Cooksey. 1991. Susceptibility tests: special tests, p. 1153-1165. In A. Balows, W. J. Hausler, Jr., K. L. Herrmann, H. D. Isenberg, and H. J. Shadomy (ed.), *Manual of clinical microbiology*, 5th ed. American Society for Microbiology, Washington, D.C.
- Sum, P. E., V. J. Lee, R. T. Testa, J. J. Hlavka, G. A. Ellestad, J. D. Bloom, Y. Gluzman, and F. P. Tally. 1993. Glycylicyclines. I. A new generation of potent antibacterial agents through modification of 9-aminotetracyclines. *J. Med. Chem.* 37:184-188.
- Sutter, V. L., D. M. Citron, M. A. C. Edelstein, and S. M. Finegold. 1985. *Wadsworth anaerobic bacteriology manual*, 4th ed. Star Publishing Co., Belmont, Calif.
- Testa, R. T., P. J. Petersen, N. V. Jacobus, P. E. Sum, V. J. Lee, and F. T. Tally. 1993. In vitro and in vivo antibacterial activities of the glycylicyclines, a new class of semisynthetic tetracyclines. *Antimicrob. Agents Chemother.* 37:2270-2277.
- Uitley, A. H. C., C. H. Collins, J. Naidoo, and K. C. George. 1988. Vancomycin resistant enterococci. *Lancet* i:57-58. (Letter.)
- Wexler, H. M., E. Molitoris, and S. M. Finegold. 1994. In vitro activities of two new glycylicyclines, *N,N*-dimethylglycylamido derivatives of minocycline and 6-demethyl-6-deoxytetracycline, against 339 strains of anaerobic bacteria. *Antimicrob. Agents Chemother.* 38:2513-2515.
- Wise, R., and J. M. Andrews. 1994. In vitro activity of two glycylicyclines. *Antimicrob. Agents Chemother.* 38:1096-1102.

In Vitro Activities of Tigecycline (GAR-936) against Multidrug-Resistant *Staphylococcus aureus* and *Streptococcus pneumoniae*

During recent years, a dramatic increase in bacterial resistance to antibiotics has been observed worldwide, both in community and nosocomial isolates, curtailing the use of many valuable antibiotics. In France, more than 50% of *Streptococcus pneumoniae* strains isolated in the community are resistant to penicillin and macrolides (8), and more than 40% of *Staphylococcus aureus* strains isolated in hospitals are resistant to β -lactams, aminoglycosides, macrolides, and fluoroquinolones, including the newer compounds ($\approx 100\%$ of the methicillin-resistant *S. aureus* [MRSA] strains) (5). *S. aureus* strains with low-level resistance to glycopeptides have been isolated in our hospital for more than 10 years (6), and resistance to quinupristin-dalfopristin (Synercid) is not uncommon due to the frequent use of oral pristinamycin in France. Most of these multidrug-resistant (MDR) bacteria are resistant to tetracyclines, emphasizing the need for new compounds.

Tigecycline (GAR-936) is the 9-*t*-butylglycylamino derivative of minocycline, a new generation of tetracyclines called glycylcyclines. These glycylcyclines overcome tetracycline resistance due to both ribosomal protection and efflux determinants (1–3, 7). The aim of this study was to assess the in vitro activity of GAR-936 against MDR *S. pneumoniae* and *S. aureus* in comparison with tetracycline, minocycline, telithromycin, linezolid, and quinupristin-dalfopristin.

Bacterial strains. A total of 133 *S. aureus* and 105 *S. pneumoniae* epidemiologically unrelated strains were selected for MIC studies.

Among the 133 *S. aureus* strains, 38 were tetracycline susceptible (15 methicillin-susceptible *S. aureus* [MSSA] and 23 MRSA strains) and 95 were tetracycline resistant (28 MSSA and 67 MRSA strains); quinupristin-dalfopristin resistance was observed in 25 strains (6 MSSA and 19 MRSA), and 11 strains (all MRSA) were considered as glycopeptide-intermediate *S. aureus* strains.

Among the 105 *S. pneumoniae* strains, 13 strains were tetracycline susceptible (6 penicillin-resistant strains), and 92 strains were resistant to tetracycline (74 penicillin-resistant strains).

Antibiotics. Standard reference powders were obtained from Wyeth Ayerst (tigecycline [GAR-936] and minocycline), Phar-

macia (linezolid), and Aventis (telithromycin, quinupristin-dalfopristin, and tetracycline).

MICs. Bacterial strains were grown overnight in brain heart infusion (BHI) broth (*S. aureus*) or a combination of BHI broth and 10% horse serum (*S. pneumoniae*) and diluted in order to obtain 10^6 CFU/ml. A twofold dilution of the antibiotics was done in Mueller-Hinton agar (MHA; for *S. aureus* strains) or a combination of MHA and 5% horse blood (for *S. pneumoniae* strains). Bacteria were deposited with a Steers replication device in order to obtain $\approx 10^3$ to 10^4 CFU/spot. All the plates were incubated for 18 h at 37° in ambient air. The MIC was considered as the lowest concentration inhibiting visible growth (or <3 colonies). Strains were classified as susceptible, intermediate, or resistant according to NCCLS criteria.

The activity of GAR-936 and other antibiotics on *S. aureus* is presented in Table 1 delineating the most remarkable resistance patterns: resistance to tetracycline, β -lactams, and quinupristin-dalfopristin. Against tetracycline-susceptible strains, the mode MIC of GAR-936 was equal to the MICs of tetracycline, quinupristin-dalfopristin, and telithromycin, lower than the MIC of linezolid, but 1 dilution higher than that of minocycline. Against tetracycline-resistant strains, GAR-936 was by far the most active compound, with a mode MIC of 0.5 $\mu\text{g/ml}$ in contrast to a mode MIC of 4 to 128 $\mu\text{g/ml}$ for other antibiotics. Moreover, there was no shift to higher GAR-936 MICs for strains resistant to tetracycline and minocycline or other antibiotics, as inferred from the very narrow MIC range (mode ± 1 dilution) despite the diversity of strains and resistance mechanisms (minocycline MICs ranged from 0.12 to 16 $\mu\text{g/ml}$). This absence of increased resistance to GAR-936—in other words, the absence of both cross-resistance and associated resistance—was unexpected since resistant mutants can be selected in vitro.

The activity against *S. pneumoniae* is presented in Table 1. Against tetracycline-susceptible strains, as noted also for tetracycline-susceptible *S. aureus* strains, the mode MIC of GAR-936 was 1 dilution lower than that of tetracycline and 1 dilution

TABLE 1. MICs of GAR-936 and other drugs for *S. aureus* and *S. pneumoniae* strains

Organism	Total no. of strains	MIC ^a (µg/ml)					
		GAR-936	TET	MIN	LIN	TEL	SYN
<i>S. aureus</i> strains							
Tetracycline susceptible	38	0.25-1 (0.5)	0.5-4 (0.5)	0.12-0.5 (0.25)	1-4 (2)	0.12-128 (0.5)	0.5-16 (0.5)
Tetracycline resistant	84	0.25-1 (0.5)	16-128 (64)	0.12-16 (4)	0.25-4 (2)	0.12-128 (128)	0.25-32 (1)
Glycopeptide intermediate	11	0.5-1 (0.5)	32-64 (32)	2-8 (4)	1-2 (2)	>128	0.5-16 (1)
<i>S. pneumoniae</i> strains							
Tetracycline susceptible	13	0.12-0.5 (0.25)	0.25-2 (0.5)	0.12-0.5 (0.12)	0.5-2 (1)	0.01-1 (0.03)	1-2 (2)
Tetracycline resistant	92	0.06-0.5 (0.12)	8-128 (64)	1-64 (8)	0.5-2 (1)	0.03-2 (0.06)	0.5-4 (1)

^a Values are the MIC ranges of the drugs for the strains; the MICs at which 50% of the strains are inhibited are given in parentheses. TET, tetracycline; MIN, minocycline; LIN, linezolid; TEL, telithromycin; SYN, quinupristin-dalfopristin.

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higher than that of minocycline. Against tetracycline-resistant *S. pneumoniae* strains, GAR-936 was 32- to 64-fold more active than minocycline, with no GAR-936 MIC exceeding 0.5 µg/ml versus a minocycline MIC of 64 µg/ml. Against penicillin-resistant strains, the MICs of GAR-936 were lower by 1 dilution than the MICs for penicillin susceptible strains.

This interesting finding, not previously reported, must be confirmed in isogenic strains, however. As with *S. aureus* strains, no shift in the MICs of GAR-936 have been observed for strains resistant to other antibiotics, particularly macrolides and fluoroquinolones. This result suggests that the risk of selection by unrelated antibiotics of multidrug-resistant strains, as frequently occurs with *S. aureus* and *S. pneumoniae* strains (4), is minimized.

These data indicate that GAR-936 is a very valuable compound and warrants further in vitro and clinical studies.

REFERENCES

1. Betriu, C., I. Rodriguez-Avial, B. A. Sanchez, M. Gomez, J. Alvarez, and J. J. Picazo. 2002. In vitro activities of tigecycline (GAR-936) against recently isolated clinical bacteria in Spain. *Antimicrob. Agents Chemother.* **46**:892-895.
2. Boucher, H. W., C. B. Wennersten, and G. M. Eliopoulos. 2000. In vitro activities of the glycylcycline GAR-936 against gram-positive bacteria. *Antimicrob. Agents Chemother.* **44**:2225-2229.
3. Chopra, I. 2001. Glycylcyclines: third-generation tetracycline antibiotics. *Curr. Opin. Pharmacol.* **1**:464-469.
4. Goldstein, F. W. 1999. Penicillin-resistant *Streptococcus pneumoniae*: selection by beta-lactam and non-beta-lactam antibiotics. *J. Antimicrob. Chemother.* **44**:141-144.
5. Leclercq, R. 2002. Staphylococci resistant to antibiotic therapy. *Ann. Fr. Anesth. Reanim.* **21**:375-383.
6. Mainardi, J. L., D. M. Shlaes, R. V. Goering, J. H. Shlaes, J. F. Acur, and F. W. Goldstein. 1995. Decreased teicoplanin susceptibility of methicillin-resistant strains of *Staphylococcus aureus*. *J. Infect. Dis.* **171**:1646-1650.
7. Petersen, P. J., N. V. Jacobus, W. J. Weiss, P. E. Sum, and R. T. Testa. 1999. In vitro and in vivo antibacterial activities of a novel glycylcycline, the 9-t-butylglycylamido derivative of minocycline (GAR-936). *Antimicrob. Agents Chemother.* **43**:738-744.
8. Schito, G. C., E. A. Debbia, and A. Marchese. 2000. The evolving threat of antibiotic resistance in Europe: new data from the Alexander Project. *J. Antimicrob. Chemother.* **46**(Suppl. T1):3-9.

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In vitro activity of tigecycline against 6792 Gram-negative and Gram-positive clinical isolates from the global Tigecycline Evaluation and Surveillance Trial (TEST Program, 2004).

Hoban DJ, Bouchillon SK, Johnson BM, Johnson JL, Dowzicky MJ; Tigecycline Evaluation and Surveillance Trial (TEST Program) Group.

International Health Management Associates, Inc, Schaumburg, IL 60173-3817, USA.
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Tigecycline, a new glycylicycline antibiotic, has shown promising in vitro activity against many common pathogens, including multidrug-resistant strains. To determine the activity of tigecycline against a broad range of pathogens from diverse populations and geographic areas, the Tigecycline Evaluation and Surveillance Trial (TEST Program) commenced in 2003. This study evaluated the activity of tigecycline and commonly used antimicrobials against 6792 clinical isolates from 40 study centers in 11 countries. Tigecycline was the most active agent tested against Gram-positive facultative species including multidrug-resistant strains. MIC90 results (microg/mL) for tigecycline against *Enterococcus faecalis*, *Enterococcus faecium*, *Staphylococcus aureus*, *Streptococcus agalactiae*, and *Streptococcus pneumoniae* were 0.12, 0.12, 0.25, and 0.25 microg/mL, respectively. Tigecycline was active against Enterobacteriaceae with an MIC90 of 1 microg/mL. *Haemophilus influenzae* was very susceptible to tigecycline with an MIC90 of only 0.25 microg/mL. *Pseudomonas aeruginosa* was the least susceptible organism tested against tigecycline. Tigecycline appears to be a promising new glycylicycline agent for the treatment of many types of pathogens with varying resistance phenotypes.

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Antimicrobial activity of tigecycline (GAR-936) tested against 3498 recent isolates of Staphylococcus aureus recovered from nosocomial and community-acquired infections
Fritsche, TR | Jones, RN
International Journal of Antimicrobial Agents [Int. J. Antimicrob. Agents]. Vol. 24, no. 6, pp. 567-571. Dec 2004.

Tigecycline is a novel 9-t-butylglycylamido derivative of minocycline that has demonstrated activity against a variety of Gram-positive and -negative bacterial pathogens. In vitro activity of tigecycline and comparator agents was determined for 3498 recent (2000-2003) strains of *Staphylococcus aureus* recovered from patients with either nosocomial or community-acquired infections. Oxacillin-susceptible and -resistant *S. aureus* from both patient populations displayed identical results for tigecycline (MIC sub(50) and MIC sub(90) results at 0.25 and 0.5 mg/L, respectively) and all strains were inhibited by 1 mg/L or less. While co-resistances to other antimicrobial classes were present in oxacillin-resistant strains, susceptibility to tigecycline remained unaffected, making the compound an attractive candidate for treatment of serious hospital as well as community-acquired staphylococcal infections.

② **Descriptors:** **Article Subject Terms** Antimicrobial activity | Antimicrobial agents | Gram-negative bacteria | Gram-positive bacteria | Hospitals | Minocycline | Oxacillin | Pathogens | tigecycline | **Article Taxonomic Terms** *Staphylococcus aureus*

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(54) Title: METHODS AND REAGENTS FOR PREVENTING BACTEREMIAS

(57) Abstract: The present invention provides methods and compositions useful for preventing bacteremia by decolonizing the intestinal tract of a patient. Although the present invention is useful for preventing bacteremia by any Gram-positive bacteria, it is particularly useful against antibiotic-resistant bacteria, such as vancomycin-resistant *Enterococcus* (VRE), methicillin-resistant *Staphylococcus aureus* (MRSA), glycopeptide intermediary susceptible *Staphylococcus aureus* (GISA), and penicillin-resistant *Streptococcus pneumoniae* (PRSP). Decolonization therapy using the methods and compositions of this invention are also useful for preventing a Gramnegative bacteremia.

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Claims

1. A preventive method comprising the steps of:

- a) identifying a patient whose intestinal tract is colonized with Gram-positive bacteria, but who does not have a bacteremia caused by said bacteria; and
- b) orally administering to said patient one or more antibiotics selected from the group consisting of: teicoplanin, daptomycin, oritavancin, dalbavancin, everminomycin, virginiamycin, quinupristin, dalbopristin, linezolid, tigecycline, pristinamycin, nisin, moenomycin, gemifloxacin, tunicamycin, cinnamycin, laspartomycin, novobiocin, ciprofloxacin, moxifloxacin, chloramphenicol, nitrofurantoin, cyclo-(Leu-Pro), fosfomycin, telithromycin, azithromycin, magainin, iseganan, BMS-284,756, L-749,345, ER-35,786, S-4661, L-786,392, MC-02479, Pep5, RP 59500, and TD-6424 in an amount and for a duration sufficient to substantially decolonize the intestinal tract of said patient of said bacteria.

2. A preventive method comprising the steps of:

- a) identifying a patient whose intestinal tract is colonized with Gram-positive bacteria, but who does not have a bacteremia caused by said bacteria; and
- b) orally administering to said patient one or more antibiotics selected from the group consisting of bacteriocins, type A lantibiotics, type B lantibiotics, liposidomycins, mureidomycins, alanoylcholines, quinolines, everminomycins, glycylicyclines, carbapenems, cephalosporins, streptogramins, oxazolidonones, tetracyclines, cyclothialidines, bioxalomycins, cationic peptides, and protegrins in an amount and for a duration sufficient to substantially decolonize the intestinal tract of said patient of said bacteria.

3. The method of claim 1 or 2, said method further comprising culturing said bacteria from a stool sample or rectal swab obtained from said patient.
4. The method of claim 1 or 2, said method further comprising nucleic acid analysis of said bacteria.
5. A preventive method comprising the step of orally administering to a patient one or more antibiotics selected from the group consisting of teicoplanin, daptomycin,oritavancin, dalbavancin, everninomycin, virginiamycin, quinupristin, dalfopristin, linezolid, tigecycline, pristnamycin, nisin, moenomycin, gemifloxacin, tunicamycin, cinnamycin, laspartomycin, novobiocin, ciprofloxacin, moxifloxacin, chloramphenicol, nitrofurantoin, cyclo-(Leu-Pro), fosfomycin, telithromycin, azithromycin, magainin, iseganan, BMS-284,756, L-749,345, ER-35,786, S-4661, L-786,392, MC-02479, Pep5, RP 59500, and TD-6424 in an amount and for a duration sufficient to substantially decolonize the intestinal tract of said patient of Gram-positive bacteria, wherein substantially all of said antibiotic is non-absorbable or partially non-absorbable, and retains antibacterial activity in the lumen of said intestinal tract.
6. The method of any of claims 1, 2, or 5, wherein said antibiotic is formulated such that substantially all of said antibiotic is non-absorbable or partially non-absorbable, and retains antibacterial activity in the lumen of the intestinal tract of said patient.
7. The method of any of claims 1, 2, or 5, wherein said bacteria are antibiotic-resistant.

8. The method of claim 7, wherein said antibiotic-resistant Gram-positive bacteria comprise bacteria of the genus *Enterococcus*.

9. The method of claim 8, wherein said bacteria are *E. faecium*, *E. faecalis*, *E. raffinosus*, *E. avium*, *E. hirae*, *E. gallinarum*, *E. casseliflavus*, *E. durans*, *E. malodoratus*, *E. mundtii*, *E. solitarius*, or *E. pseudoavium*.

10. The method of claim 9, wherein said bacteria is resistant to one or more antibiotics selected from the group consisting of vancomycin, teicoplanin, daptomycin, oritavancin, dalbavancin, everninomycin, quinupristin/dalfopristin, linezolid, and tigecycline.

11. The method of claim 9, wherein said bacteria is resistant to one or more antibiotics selected from the group consisting of glycopeptides, everninomycins, streptogramins, lipopeptides, oxazolidonones, bacteriocins, type A lantibiotics, type B lantibiotics, liposidomycins, mureidomycins, and alanoylcholines.

12. The method of claim 7, wherein said antibiotic-resistant Gram-positive bacteria comprise bacteria of the genus *Staphylococcus*.

13. The method of claim 12, wherein said bacteria is *S. aureus*, *S. epidermidis*, *S. hominis*, *S. saprophyticus*, *S. hemolyticus*, *S. capitis*, *S. auricularis*, *S. lugdenis*, *S. warneri*, *S. saccharolyticus*, *S. caprae*, *S. pasteurii*, *S. schleiferi*, *S. xylosus*, *S. cohnii*, or *S. simulans*.

14. The method of claim 13, wherein said bacteria is resistant to one or more antibiotics selected from the group consisting of methicillin, teicoplanin, daptomycin, oritavancin, dalbavancin, everninomycin, quinupristin/dalfopristin, linezolid, and tigecycline.

15. The method of claim 13, wherein said bacteria is resistant to one or more antibiotics selected from the group consisting of glycopeptides, everninomycins, streptogramins, lipopeptides, oxazolidonones, bacteriocins, type A lantibiotics, type B lantibiotics, liposidomycins, mureidomycins, and alanoylcholines.

16. The method of claim 7, wherein said antibiotic-resistant Gram-positive bacteria comprise bacteria of the genus *Streptococcus*.

17. The method of claim 16, wherein said bacteria is *S. pyogenes*, *S. agalactiae*, *S. pneumoniae*, *S. bovis*, or *S. viridans*.

18. The method of claim 17, wherein said bacteria is resistant to one or more antibiotics selected from the group consisting of penicillin, teicoplanin, daptomycin, oritavancin, dalbavancin, everninomycin, quinupristin/dalfopristin, linezolid, and tigecycline.

19. The method of claim 17, wherein said bacteria is resistant to one or more antibiotics selected from the group consisting of glycopeptides, everninomycins, streptogramins, lipopeptides, oxazolidonones, bacteriocins, type A lantibiotics, type B lantibiotics, liposidomycins, mureidomycins, β -lactam antibiotics, and alanoylcholines,

20. The method of any of claims 1, 2, or 5, wherein said patient is neutropenic.

21. The method of any of claims 1, 2, or 5, wherein said patient is within 14 days of receiving chemotherapy or radiation therapy in preparation for autologous or allogeneic hematopoietic stem cell transplant, bone marrow transplant or solid organ transplant.

22. The method of any of claims 1, 2, or 5, wherein said patient is within 14 days of receiving antineoplastic radiation or chemotherapy.

23. The method of any of claims 1, 2, or 5, wherein said patient has or is at risk for enteritis, colitis, or mucositis of the intestinal tract.

24. The method of any of claims 1, 2, or 5, wherein said patient is diagnosed as having a human immunodeficiency virus (HIV) infection, or has acquired immunodeficiency syndrome (AIDS).

25. The method of any of claims 1, 2, or 5, wherein said patient is diagnosed as having chronic renal insufficiency.

26. A preventive method comprising the steps of:

a) identifying a patient whose intestinal tract is colonized with Gram-negative bacteria, but who does not have a bacteremia caused by said bacteria; and

b) orally administering to said patient one or more antibiotics selected from the group consisting of: teicoplanin, daptomycin, oritavancin, dalbavancin, everninomycin, virginiamycin, quinupristin, dalbopristin, linezolid, tigecycline, pristinamycin, nisin, moenomycin, gemifloxacin, tunicamycin, cinnamycin, laspartomycin, novobiocin, ciprofloxacin, moxifloxacin, chloramphenicol, nitrofurantoin, cyclo-(Leu-Pro), fosfomycin, telithromycin, azithromycin, magainin, iseganan, BMS-284,756, L-749,345, ER-35,786, S-4661, L-786,392, MC-02479, Pep5, RP 59500, and TD-6424 in an amount and for a duration sufficient to substantially decolonize the intestinal tract of said patient of said bacteria.

27. The methods of claim 26, wherein said bacteria is selected from the group consisting of *S. typhimurium*, *S. enteritidis*, *S. newport*, *S. anatum*, *S. typhi*, *S. paratyphi*, *S. schottmuelleri*, *S. hirscheidii*, *S. dysenteriae*, *S. flexneri*, *S. boydii*, *S. sonnei*, *Y. enterocolitica*, *Y. pestis*, *P. mirabilis*, *P. vulgaris*, *Klebsiella pneumoniae*, *Vibrio cholerae*, and *Campylobacter jejuni*.

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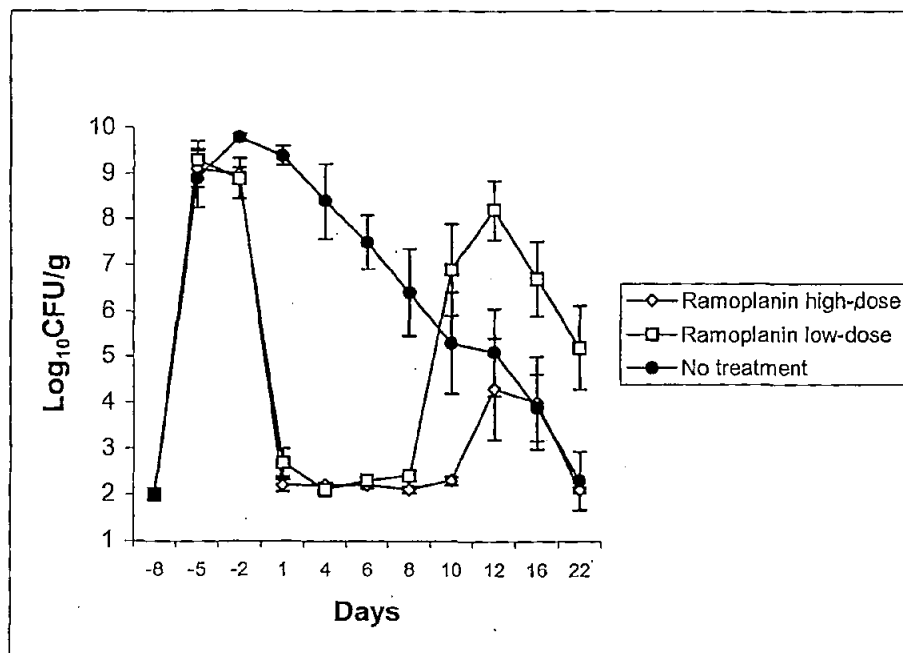
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[Continued on next page]

(54) Title: METHODS FOR REDUCING OR PREVENTING TRANSMISSION OF NOSOCOMIAL PATHOGENS IN A HEALTH CARE FACILITY



(57) Abstract: The present invention provides methods and compositions useful for reducing or preventing the transmission of nosocomial pathogens or an epidemic of nosocomial pathogens in a health care facility by decolonizing the gastro-intestinal tract, skin, or nasal passage of carriers and by preventing colonization of individuals at risk who may serve as transmission vehicles or vectors to other individuals.

Claims

1. A method for reducing or preventing the transmission of a nosocomial pathogen, said method comprising the steps of:
 - a. identifying a carrier who is colonized with a nosocomial pathogen; and
 - b. administering an antibiotic, in an amount and for a duration sufficient to prevent colonization or infection by said pathogen, to a population of individuals at risk of being colonized or infected by said pathogen from said carrier.
2. The method of claim 1, wherein the gastrointestinal tract of said carrier is colonized with said nosocomial pathogen and said antibiotic is orally administered to said carrier.
3. The method of claim 1, wherein the skin of said carrier is colonized with said nosocomial pathogen and said antibiotic is topically administered to said carrier.
4. The method of claim 1, wherein the nasal mucosa or sinus of said carrier is colonized with said nosocomial pathogen, and said antibiotic is intranasally administered to said carrier.
5. The method of claim 1, wherein said antibiotic is orally administered to said population of individuals.
6. The method of claim 1, wherein substantially all of said antibiotic is non-absorbable or partially non-absorbable when administered.

7. The method of claim 1, wherein said antibiotic is selected from the group consisting of teicoplanin, daptomycin, oritavancin, dalbavancin, everninomycin, quinupristin/dalfopristin, linezolid, tigecycline, colistin, amphotericin, nystatin, iseganan, and ramoplanin.

8. The method of claim 7, wherein said antibiotic is ramoplanin.

9. The method of claim 1, wherein said antibiotic is selected from the group consisting of polymyxins, aminoglycosides, glycopeptides, everninomycins, streptogramins, lipopeptides, oxazolidonones, bacteriocins, type A lantibiotics, type B lantibiotics, liposidomycins, mureidomycins, and alanoylcholines.

10. The method of claim 1, wherein said pathogen is a Gram-positive bacterium.

11. The method of claim 1, wherein said carrier has a bacteremia.

12. The method of claim 1, wherein at least one member of said population being treated is not tested for the presence of said pathogen.

13. The method of claim 1, wherein said carrier or a member of said population being treated is a patient, an employee, or a visitor in a health care facility.

14. The method of claim 1, wherein said carrier or a member of said population is a doctor, nurse, orderly, medical student, physical therapist, health care administrator, visiting nurse, food service personnel, or janitor; or works in an intensive care unit, surgical unit, or geriatric ward.

15. The method of claim 1, wherein said carrier or a member of said population has received broad-spectrum antibiotic therapy for at least one week within the previous month or is receiving concurrent broad-spectrum antibiotic therapy.

16. The method of claim 1, wherein said carrier or a member of said population is immunocompromised.

17. The method of claim 1, wherein said carrier or a member of said population has neutropenia, a human immunodeficiency virus (HIV) infection, acquired immunodeficiency syndrome (AIDS), or is within 14 days of receiving chemotherapy or radiation therapy in preparation for autologous or allogeneic hematopoietic stem cell transplant, bone marrow transplant, or solid organ transplant, or as a part of antineoplastic therapy.

18. The method of claim 16, wherein said carrier or a member of said population has been receiving immunosuppressive therapy for at least seven days.

19. The method of claim 18, wherein said immunosuppressive therapy comprises a steroid.

20. The method of claim 1, wherein a member of said population has or is at risk for enteritis, colitis, typhlitis, or mucositis of the gastro-intestinal tract.

21. The method of claim 1, wherein said pathogen is a bacterium that is antibiotic-resistant.

22. The method of claim 21, wherein said bacterium is of the genus *Enterococcus*.

23. The method of claim 22, wherein said bacterium is *E. faecium*, *E. faecalis*, *E. raffinosus*, *E. avium*, *E. hirae*, *E. gallinarum*, *E. casseliflavus*, *E. durans*, *E. malodoratus*, *E. mundtii*, *E. solitarius*, or *E. pseudoavium*.

24. The method of claim 22, wherein said bacterium is resistant to vancomycin.

25. The method of claim 22, wherein said bacterium is resistant to one or more antibiotics selected from the group consisting of teicoplanin, daptomycin, oritavancin, dalbavancin, everninomycin, quinupristin/dalfopristin, linezolid, tigecycline, glycopeptides, everninomycins, streptogramins, lipopeptides, oxazolidonones, bacteriocins, type A lantibiotics, type B lantibiotics, liposidomycins, mureidomycins, and alanoylcholines.

26. The method of claim 21, wherein said bacterium is of the genus *Staphylococcus*.

27. The method of claim 26, wherein said bacterium is *S. aureus*, *S. epidermidis*, *S. hominis*, *S. saprophyticus*, *S. hemolyticus*, *S. capitis*, *S. auricularis*, *S. lugdenis*, *S. warneri*, *S. saccharolyticus*, *S. caprae*, *S. pasteurii*, *S. schleiferi*, *S. xylosus*, *S. cohnii*, or *S. simulans*.

28. The method of claim 26, wherein said bacterium is resistant to methicillin.

29. The method of claim 26, wherein said bacterium is resistant to one or more antibiotics selected from the group consisting of teicoplanin, daptomycin, oritavancin, dalbavancin, everninomycin, quinupristin/dalfopristin, linezolid, tigecycline, glycopeptides, everninomycins, streptogramins, lipopeptides, oxazolidonones, bacteriocins, type A lantibiotics, type B lantibiotics, liposidomycins, mureidomycins, and alanoylcholines.

30. The method of claim 21, wherein said bacterium is of the genus *Streptococcus*.

31. The method of claim 30, wherein said bacterium is *S. pyogenes*, *S. agalactiae*, *S. pneumoniae*, *S. bovis*, or *S. viridans*.

32. The method of claim 30, wherein said bacterium is resistant to penicillin.

33. The method of claim 30, wherein said bacterium is resistant to one or more antibiotics selected from the group consisting of teicoplanin, daptomycin, oritavancin, dalbavancin, everninomycin, quinupristin/dalfopristin, linezolid, tigecycline, glycopeptides, everninomycins, streptogramins, lipopeptides, oxazolidonones, bacteriocins, type A lantibiotics, type B lantibiotics, liposidomycins, mureidomycins, and alanoylcholines.

34. The method of claim 21, wherein said bacterium is *Clostridium difficile* or *Clostridium perfringens*.

35. The method of claim 8, wherein said antibiotic is ramoplanin, wherein said ramoplanin is orally administered one to six times daily at a dosage of between about 50 mg and 400 mg.

36. The method of claim 35, wherein said ramoplanin is administered twice daily at a dosage of between about 200 mg and 400 mg.

37. The method of claim 8, wherein said ramoplanin is administered topically or intranasally one to six times daily at a dose of 0.1% to 90% by weight.

38. The method of claim 1, wherein said population of individuals is further administered a second antibiotic having activity against Gram-negative bacteria.

39. A method for reducing or preventing the transmission of a nosocomial pathogen, said method comprising the steps of:

- a. identifying a fomite that is contaminated with a nosocomial pathogen; and
- b. administering an antibiotic, in an amount and for a duration sufficient to prevent colonization or infection by said pathogen, to a population of individuals at risk of being colonized or infected by said.

40. The method of claim 39, wherein said fomite has been exposed to a carrier who is colonized with said nosocomial pathogen.

41. The method of claim 39, wherein said identifying step (a) comprises contacting a candidate fomite with a culture swab and culturing said swab to identify a nosocomial pathogen.

42. The method of claim 39, wherein said identifying step (a) comprises the polymerase chain reaction.

43. The method of claim 39, wherein said antibiotic is orally administered to said population of individuals.

44. The method of claim 39, wherein substantially all of said antibiotic is non-absorbable or partially non-absorbable when administered.

45. The method of claim 39, wherein said antibiotic is selected from the group consisting of teicoplanin, daptomycin, oritavancin, dalbavancin, everninomycin, quinupristin/dalfopristin, linezolid, tigecycline, colistin, amphotericin, nystatin, iseganan, and ramoplanin.

46. The method of claim 39, wherein said antibiotic is ramoplanin.

47. The method of claim 39, wherein said antibiotic is selected from the group consisting of polymixins, aminoglycosides, glycopeptides, everninomycins, streptogramins, lipopeptides, oxazolidonones, bacteriocins, type A lantibiotics, type B lantibiotics, liposidomycins, mureidomycins, and alanoylcholines.

48. The method of claim 39, wherein said pathogen is a Gram-positive bacterium.

49. The method of claim 39, wherein at least one member of said population being treated is not tested for the presence of said pathogen.

50. The method of claim 40, wherein said carrier has not been identified.

51. The method of claim 39, wherein a member of said population being treated is a patient, employee, or visitor in a health care facility.

52. The method of claim 39, wherein a member of said population being treated is a doctor, nurse, orderly, medical student, physical therapist, health care administrator, visiting nurse, food service personnel, or janitor, or works in an intensive care unit, surgical unit, or geriatric ward.

53. The method of claim 39, wherein said fomite bedding or bandages.

54. The method of claim 39, wherein said fomite is an environmental surface.

55. The method of claim 39, wherein a member of said population is immunocompromised.

56. The method of claim 39, wherein said carrier or a member of said population has neutropenia, a human immunodeficiency virus (HIV) infection, acquired immunodeficiency syndrome (AIDS), or is within 14 days of receiving chemotherapy or radiation therapy in preparation for autologous or allogeneic hematopoietic stem cell transplant, bone marrow transplant, or solid organ transplant, or as a part of antineoplastic therapy.

57. The method of claim 55, wherein said member is has been receiving immunosuppressive therapy for at least seven days.

58. The method of claim 57, wherein said immunosuppressive therapy comprises a steroid.

59. The method of claim 39, wherein a member of said population has or is at risk for enteritis, colitis, typhlitis, or mucositis of the gastro-intestinal tract.

60. The method of claim 39, wherein said pathogen is a bacterium that is antibiotic-resistant.

61. The method of claim 60, wherein said bacterium is of the genus *Enterococcus*.

62. The method of claim 61, wherein said bacterium is *E. faecium*, *E. faecalis*, *E. raffinosus*, *E. avium*, *E. hirae*, *E. gallinarum*, *E. casseliflavus*, *E. durans*, *E. malodoratus*, *E. mundtii*, *E. solitarius*, or *E. pseudoavium*.

63. The method of claim 61, wherein said bacterium is resistant to vancomycin.

64. The method of claim 61, wherein said bacterium is resistant to one or more antibiotics selected from the group consisting of teicoplanin, daptomycin, oritavancin, dalbavancin, everninomycin, quinupristin/dalfopristin, linezolid, tigecycline, glycopeptides, everninomycins, streptogramins, lipopeptides, oxazolidonones, bacteriocins, type A lantibiotics, type B lantibiotics, liposidomycins, mureidomycins, and alanoylcholines.

65. The method of claim 60, wherein said bacterium is of the genus *Staphylococcus*.

66. The method of claim 65, wherein said bacterium is *S. aureus*, *S. epidermidis*, *S. hominis*, *S. saprophyticus*, *S. hemolyticus*, *S. capitis*, *S. auricularis*, *S. lugdenis*, *S. warneri*, *S. saccharolyticus*, *S. caprae*, *S. pasteurii*, *S. schleiferi*, *S. xylosus*, *S. cohnii*, or *S. simulans*.

67. The method of claim 65, wherein said bacterium is resistant to methicillin.

68. The method of claim 65, wherein said bacterium is resistant to one or more antibiotics selected from the group consisting of teicoplanin, daptomycin, oritavancin, dalbavancin, everninomycin, quinupristin/dalfopristin, linezolid, tigecycline, glycopeptides, everninomycins, streptogramins, lipopeptides, oxazolidonones, bacteriocins, type A lantibiotics, type B lantibiotics, liposidomycins, mureidomycins, and alanoylcholines.

69. The method of claim 60, wherein said bacterium is of the genus *Streptococcus*.

70. The method of claim 69, wherein said bacterium is *S. pyogenes*, *S. agalactiae*, *S. pneumoniae*, *S. bovis*, or *S. viridans*.

71. The method of claim 69, wherein said bacterium is resistant to penicillin.

72. The method of claim 69, wherein said bacterium is resistant to one or more antibiotics selected from the group consisting of teicoplanin, daptomycin, oritavancin, dalbavancin, everninomycin, quinupristin/dalfopristin, linezolid, tigecycline, glycopeptides, everninomycins, streptogramins, lipopeptides, oxazolidonones, bacteriocins, type A lantibiotics, type B lantibiotics, liposidomycins, mureidomycins, and alanoylcholines.

73. The method of claim 60, wherein said bacterium is *Clostridium difficile* or *Clostridium perfringens*.

74. The method of claim 46, wherein said antibiotic is ramoplanin, wherein said ramoplanin is orally administered one to six times daily at a dosage of between about 50 mg and 400 mg.

75. The method of claim 74, wherein said ramoplanin is administered twice daily at a dosage of between about 200 mg and 400 mg.

76. The method of claim 46, wherein said ramoplanin is administered topically or intranasally one to six times daily at a dose of 0.1% to 90% by weight.

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(54) Title: **USE OF TIGECYCLINE, ALONE, OR IN COMBINATION WITH RIFAMPIN TO TREAT OSTEOMYELITIS
AND/OR SEPTIC ARTHRITIS**

(57) Abstract: The present invention is directed to a method for treating bone or bone marrow infections, joint infection or infection of the tissues surrounding the joint by administration of the antibiotic tigecycline alone or in combination with a rifamycin antibiotic. In a preferred embodiment the bone or bone marrow infection causes osteomyelitis. In another embodiment the joint infection or infection of the tissues surrounding the joint causes septic arthritis. The invention is also directed to manufacture of a medicament for treatment of bone and/or bone marrow infections, or joint infections and/or infections in tissues surrounding the joint with tigecycline alone or in combination with rifampin.

What is claimed is:

1. A method of treating an infection in bone or bone marrow in a mammal comprising administering to the mammal a pharmacologically effective amount of tigecycline.
5
2. The method of claim 1 further comprising administering an antimicrobial agent selected from the group consisting of rifamycin, rifampin, rifapentine, rifaximin, or streptovaricin.
3. The method of claim 2 where the antimicrobial is rifampin.
- 10 4. The method of claim 1, 2 or 3 where the infection is comprised of a pathogen selected from the group consisting of gram negative bacteria, gram positive bacteria, anaerobic bacteria, and aerobic bacteria.
5. The method of claim 4 where the pathogen is selected from the group consisting of *Staphylococcus*, *Acinetobacter*, *Mycobacterium*, *Haemophilus*, *Salmonella*, *Streptococcus*,
15 *Enterobacteriaceae*, *Enterococcus*, *Escherichia*, *Pseudomonas*, *Neisseria*, *Rickettsia*, *Pneumococci*, *Prevotella*, *Peptostreptococci*, *Bacteroides*, *Legionella*, beta-haemolytic streptococci, group B streptococcus and *spirochaetes*.
6. The method of claim 5 wherein the infection is comprised of *Neisseria*, *Mycobacterium*, *Staphylococcus*, and *Haemophilus*.
- 20 7. The method of claim 6 wherein the infection is comprised of *Neisseria meningitidis*, *Mycobacterium tuberculosis*, *Mycobacterium leprae*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, or *Haemophilus influenzae*.
8. The method of claim 4 where the pathogen exhibits antibiotic resistance.
9. The method of claim 8 where the antibiotic resistance is selected from the group
25 consisting of methicillin resistance, glycopeptide resistance, tetracycline resistance, oxytetracycline resistance, doxycycline resistance; chlortetracycline resistance, minocycline resistance minocycline resistance, glycylcycline resistance, cephalosporin resistance, ciprofloxacin resistance, nitrofurantoin resistance, trimethoprim-sulfa

resistance, piperacillin/tazobactam resistance, moxifloxacin resistance, vancomycin resistance, teicoplanin resistance, penicillin resistance, and macrolide resistance.

10. The method of claim 9 where the glycopeptide resistance is vancomycin resistance.
11. The method of claim 5 where the pathogen is selected from the group consisting of
5 *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Streptococcus pneumoniae*, or *Streptococcus pyogenes*.
12. The method of claim 11 where the infection is comprised of *Staphylococcus aureus*.
13. The method of claim 12 where the *Staphylococcus aureus* exhibits an antibiotic
10 resistance selected from the group consisting of glycopeptide resistance, tetracycline resistance, minocycline resistance, methicillin resistance, vancomycin resistance and resistance to a glycyclcycline antibiotic other than tigecycline.
14. The method of claim 5 where the infection is comprised of *Acinetobacter baumannii*.
- 15 15. The method of claim 14 where the *Acinetobacter baumanii* exhibits an antibiotic resistance selected from the group consisting of cephalosporin resistance, ciprofloxacin resistance, nitrofurantoin resistance, trimethoprim-sulfa resistance, and piperacillin/tazobactam resistance.
16. The method of claim 5 where the infection is comprised of *Mycobacterium*
20 *abscessus*.
17. The method of claim 16 where the *Mycobacterium abscessus* exhibits moxifloxacin resistance.
18. The method of claim 5 where the infection is comprised of *Haemophilus influenzae*.
- 25 19. The method of claim 5 where the infection is comprised of *Enterococcus faecium*.
20. The method of claim 5 where the infection is comprised of *Escherichia coli*.

21. The method of claim 5 where the infection is comprised of *Neisseria gonorrhoeae*.
22. The method of claim 5 where the infection is comprised of *Rickettsia prowazekii*, *Rickettsia typhi*, or *Rickettsia rickettsii*.
23. The method of claim 4 wherein the infection causes osteomyelitis.
- 5 24. A method of treating a joint infection or an infection of surrounding tissues of the joint in a mammal comprising administering to the mammal a pharmacologically effective amount of tigecycline.
25. The method of claim 1 further comprising administering an antimicrobial agent selected from the group consisting of rifamycin, rifampin, rifapentine, rifaximin, or streptovaricin.
- 10 26. The method of claim 25 where the antimicrobial is rifampin.
27. The method of claim 24, 25 or 26 where the infection is comprised of a pathogen selected from the group consisting of gram negative bacteria, gram positive bacteria, anaerobic bacteria, and aerobic bacteria.
- 15 28. The method of claim 27 where the pathogen is selected from the group consisting of *Staphylococcus*, *Acinetobacter*, *Mycobacterium*, *Haemophilus*, *Salmonella*, *Streptococcus*, *Enterobacteriaceae*, *Enterococcus*, *Escherichia*, *Pseudomonas*, *Neisseria*, *Rickettsia*, *Pneumococci*, *Prevotella*, *Peptostreptococci*, *Bacteroides*, *Legionella*, beta-haemolytic streptococci, group B streptococcus and *spirochaetes*.
- 20 29. The method of claim 28 wherein the infection is comprised of *Neisseria*, *Mycobacterium*, *Staphylococcus*, and *Haemophilus*.
30. The method of claim 29 wherein the infection is comprised of *Neisseria meningitidis*, *Mycobacterium tuberculosis*, *Mycobacterium leprae*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, or *Haemophilus influenzae*.
- 25 31. The method of claim 27 where the pathogen exhibits antibiotic resistance.

32. The method of claim 31 where the antibiotic resistance is selected from the group consisting of methicillin resistance, glycopeptide resistance, tetracycline resistance, oxytetracycline resistance, doxycycline resistance; chlortetracycline resistance, minocycline resistance minocycline resistance, glycylcycline resistance, cephalosporin resistance, ciprofloxacin resistance, nitrofurantoin resistance, trimethoprim-sulfa resistance, piperacillin/tazobactam resistance, moxifloxacin resistance, vancomycin resistance, teicoplanin resistance, penicillin resistance, and macrolide resistance.
33. The method of claim 32 where the glycopeptide resistance is vancomycin resistance.
34. The method of claim 28 where the pathogen is selected from the group consisting of *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Streptococcus pneumoniae*, or *Streptococcus pyogenes*.
35. The method of claim 34 where the infection is comprised of *Staphylococcus aureus*.
36. The method of claim 35 where the *Staphylococcus aureus* exhibits an antibiotic resistance selected from the group consisting of glycopeptide resistance, tetracycline resistance, minocycline resistance, methicillin resistance, vancomycin resistance and resistance to a glycylcycline antibiotic other than tigecycline.
37. The method of claim 28 where the infection is comprised of *Acinetobacter baumannii*.
38. The method of claim 37 where the *Acinetobacter baumannii* exhibits an antibiotic resistance selected from the group consisting of cephalosporin resistance, ciprofloxacin resistance, nitrofurantoin resistance, trimethoprim-sulfa resistance, and piperacillin/tazobactam resistance.
39. The method of claim 28 where the infection is comprised of *Mycobacterium abscessus*.
40. The method of claim 39 where the *Mycobacterium abscessus* exhibits moxifloxacin resistance.

41. The method of claim 28 where the infection is comprised of a pathogen selected from the group consisting of *Haemophilus influenzae*, *Enterococcus faecium*, *Escherichia coli*, *Neisseria gonorrhoeae*, *Rickettsia prowazekii*, *Rickettsia typhi*, or *Rickettsia rickettsii*.
- 5 42. The method of claim 27 wherein the joint infection or infection of the surrounding tissues of the joint cause septic arthritis.
43. Use of a pharmacologically effective amount of tigecycline for treating bone, bone marrow or joint infections in a mammal.
44. Use of a pharmacologically effective amount of tigecycline and an antimicrobial
10 agent selected from the group consisting of rifamycin, rifampin, rifapentine, rifaximin, or streptovaricin for treating bone, bone marrow or joint infections in a mammal.
45. Use of a pharmacologically effective amount of tigecycline for manufacture of a medicament for treatment of bone, bone marrow or joint infections in a mammal.
46. Use of a pharmacologically effective amount of tigecycline and an antimicrobial
15 agent selected from the group consisting of rifamycin, rifampin, rifapentine, rifaximin, or streptovaricin for manufacture of a medicament for treatment of bone, bone marrow or joint infections in a mammal.
47. The use of claim 43 - 45, wherein the bone or bone marrow infection cause osteomyelitis.
- 20 48. The use of claim 43 - 45, wherein the joint infection or infection of the tissues surrounding the joint cause septic arthritis.



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(54) **METHODS AND REAGENTS FOR
TREATING INFECTIONS OF CLOSTRIDIUM
DIFFICILE AND DISEASES ASSOCIATED
THEREWITH**

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(57) **ABSTRACT**

The invention features methods and compositions including
rifalazil for treating *Clostridium difficile*.

TABLE 1-continued

MICs ($\mu\text{g/mL}$) for rifalazil and metronidazole on 31 strains of *C. difficile*.

Sample #	Organism ID	Metronidazole	ABI1648	Identity
16	<i>C. difficile</i> 154	0.25	≤ 0.0005	Not typable
17	<i>C. difficile</i> 166	0.5	0.001	Different
18	<i>C. difficile</i> 199	0.25	≤ 0.0005	Different
19	<i>C. difficile</i> 65C	0.5	0.002	Not typable
20	<i>C. difficile</i> 5-5-03	0.5	0.002	Not typable
21	<i>C. difficile</i> 6-5-03	0.5	0.002	Different
22	<i>C. difficile</i> 7-5-03	0.25	0.002	Different
23	<i>C. difficile</i> 11-5-03	0.5	0.001	Not typable
24	<i>C. difficile</i> 13-5-03	0.5	≤ 0.0005	Different
25	<i>C. difficile</i> 14-5-03	0.5	0.001	Not typable
26	<i>C. difficile</i> 16-5-03	0.5	0.008	Different
27	<i>C. difficile</i> 17-5-03	0.25	0.002	Different
28	<i>C. difficile</i> 19-5-03	0.5	0.002	Different
29	<i>C. difficile</i> 22-5-03	0.25	0.001	Different
30	<i>C. difficile</i> 29-5-03	0.5	> 0.5	Different
31	QC <i>C. diff</i> ATCC 43255	0.5	0.002	Different

*It should be noted that Fujii et al., (Antimicrob. Agents Chemother. 38:1118-1122, 1994) obtained a value of 1.0 for this organism, a value which differs from that described in Table 1.

[0051] These data demonstrate that rifalazil was much more effective than metronidazole against almost all 31 strains of *C. difficile*. MICs for rifalazil were in the range of 0.0005 to 0.5 $\mu\text{g/mL}$ while MICs for metronidazole were in the range of 0.25 to 0.5 $\mu\text{g/mL}$. Furthermore, identity analyses of these strains were performed, where possible, and indicate that the strains of *C. difficile* tested were not identical.

[0052] Strains 80 and 29-5-30 appeared to be less susceptible to metronidazole and rifalazil and therefore indicate the need for combination treatments described herein for the effective treatment of some strains of *C. difficile*.

Other Embodiments

[0053] All publications and patents mentioned in the above specification are herein incorporated by reference. Various modifications and variations of the described method and system of the invention will be apparent to those skilled in the art without departing from the scope and spirit of the invention. Although the invention has been described in connection with specific preferred embodiments, it should be understood that the invention as claimed should not be unduly limited to such specific embodiments. Indeed, various modifications of the described modes for carrying out the invention that are obvious to those skilled in microbiology or related fields are intended to be within the scope of the invention.

What is claimed is:

1. A method for treating a subject having an infection of *Clostridium difficile* or preventing an infection of *Clostridium difficile* in said subject, said method comprising administering to said subject an effective amount of rifalazil.
2. The method of claim 1, wherein said rifalazil is administered in an amount between 0.01 and 1000 mg/day.
3. The method of claim 2, wherein said rifalazil is administered in an amount between 1 and 100 mg/day.
4. The method of claim 3, wherein said rifalazil is administered in an amount between 1 and 50 mg/day.

5. The method of claim 4, wherein said rifalazil is administered in an amount between 5 and 25 mg/day.

6. The method of claim 1, wherein said rifalazil is administered for one to fourteen days.

7. The method of claim 6, wherein said rifalazil is administered for three to seven days.

8. The method of claim 1, wherein said rifalazil is administered as a single dose.

9. The method of claim 1, wherein said rifalazil is administered at an initial dose of between 5 and 100 mg, followed by subsequent doses of between 1 and 50 mg for three to seven days.

10. The method of claim 1, wherein said infection of *Clostridium difficile* comprises a strain of *Clostridium difficile* that is resistant to one or more antibiotics selected from the group consisting of vancomycin, rifampicin, rifabutin, rifapentine, rifaximin, and metronidazole.

11. The method of claim 1, wherein said rifalazil is administered orally, intravenously, subcutaneously, or rectally.

12. The method of claim 1, further comprising administering to said subject an agent that binds *Clostridium difficile* toxin A or toxin B.

13. The method of claim 1, further comprising administering to said subject one or more antibiotics selected from the group consisting of β -lactams, β -lactamase inhibitors, aminoglycosides, tetracyclines, lipopeptides, macrolides, ketolides, lincosamides, streptogramins, sulphonamides, oxazolidinones, quinolones, rifamycins, glycopeptides, metronidazole, garenoxacin, ramoplanin, faropenem, polymyxin, tigecycline, AZD2563, and trimethoprim.

14. The method of claim 13, wherein said β -lactam is selected from the group consisting of penicillins, cephalosporins, carbapenams, and monobactams.

15. The method of claim 14, wherein said penicillin is selected from the group consisting of penicillin G, penicillin V, methicillin, oxacillin, cloxacillin, dicloxacillin, nafcillin, ampicillin, amoxicillin, carbenicillin, ticarcillin, mezlocillin, piperacillin, azlocillin, and temocillin.

16. The method of claim 14, wherein said cephalosporin is selected from the group consisting of cephalothin, cephapirin, cephadrine, cephaloridine, cefazolin, cefamandole, cefuroxime, cephalexin, cefprozil, cefaclor, loracarbef, cefoxitin, cefmoxazole, cefotaxime, ceftizoxime, ceftinaxone, cefoperazone, ceftazidime, cefixime, cefpodoxime, cefibuten, cefdinir, cefpirome, cefepime, BAL5788, and BAI 9141.

17. The method of claim 14, wherein said carbapenam is selected from the group consisting of imipenem, eripenem, and meropenem.

18. The method of claim 14, wherein said monobactam is streptomycin.

19. The method of claim 13, wherein said β -lactamase inhibitor is selected from the group consisting of clavulanate, sulbactam, and tazobactam.

20. The method of claim 13, wherein said aminoglycoside is selected from the group consisting of streptomycin, neomycin, kanamycin, paromycin, gentamicin, tobramycin, amikacin, netilmicin, spectinomycin, sisomicin, dibekalin, and isepamicin.

21. The method of claim 20, wherein said aminoglycoside is gentamicin.

22. The method of claim 13, wherein said tetracycline is selected from the group consisting of tetracycline, chlortet-

racycline, demeclocycline, minocycline, oxytetracycline, methacycline, and doxycycline.

23. The method of claim 13, wherein said lipopeptide is daptomycin.

24. The method of claim 13, wherein said macrolide is selected from the group consisting of erythromycin, azithromycin, and clarithromycin.

25. The method of claim 24, wherein said macrolide is azithromycin.

26. The method of claim 13, wherein said ketolide is selected from the group consisting of telithromycin and ABT-773.

27. The method of claim 13, wherein said lincosamide is selected from the group consisting of lincomycin and clindamycin.

28. The method of claim 13, wherein said streptogramin is selected from the group consisting of quinupristin and dalbapristin.

29. The method of claim 13, wherein said sulphonamide is selected from the group consisting of sulphanilamide, para-aminobenzoic acid, sulfadiazine, sulfisoxazole, sulfamethoxazole, and sulfathalidine.

30. The method of claim 13, wherein said oxazolidinone is linezolid.

31. The method of claim 13, wherein said quinolone is selected from the group consisting of nalidixic acid, oxolinic acid, norfloxacin, perfloracin, enoxacin, ofloxacin, ciprofloxacin, temafloxacin, lomefloxacin, fleroxacin, grepafloxacin, sparfloxacin, trovafloxacin, clinafloxacin, gatifloxacin, moxifloxacin, gemifloxacin, and sitafloxacin.

32. The method of claim 31, wherein said quinolone is ciprofloxacin.

33. The method of claim 13, wherein said rifamycin is selected from the group consisting of rifampicin, rifabutin, rifapentine, and rifaximin.

34. The method of claim 13, wherein said antibiotic is metronidazole.

35. The method of claim 13, wherein said glycopeptide is selected from the group consisting of vancomycin, oritavancin, dalbavancin, and teicoplanin.

36. The method of claim 35, wherein said glycopeptide is teicoplanin.

37. The method of claim 35, wherein said glycopeptide is vancomycin.

38. The method of claim 37, wherein said rifalazil and vancomycin are administered simultaneously.

39. The method of claim 37, wherein said rifalazil and vancomycin are administered sequentially.

40. The method of claim 37, wherein said rifalazil and vancomycin are administered within fourteen days of each other.

41. The method of claim 40, wherein said rifalazil and vancomycin are administered within five days of each other.

42. The method of claim 41, wherein said rifalazil and vancomycin are administered within three days of each other.

43. The method of claim 42, wherein said rifalazil and vancomycin are administered within twenty-four hours of each other.

44. The method of claim 37, wherein at least one of said rifalazil and said vancomycin is administered orally.

45. The method of claim 37, wherein both of said rifalazil and said vancomycin are administered orally.

46. The method of claim 37, wherein said vancomycin is administered in an amount between 125 and 2000 mg per day.

47. The method of claim 46, wherein said vancomycin is administered in an amount between 500 and 2000 mg per day.

48. A method of treating a subject having an infection of *Clostridium difficile* or preventing an infection of *Clostridium difficile* in said subject, said method comprising administering to said subject a composition comprising rifalazil and vancomycin.

49. The method of claim 48, wherein said composition is suitable for oral administration.

50. The method of claim 48, wherein said composition is suitable for intravenous administration.

51. The method of claim 48, wherein said rifalazil is in a unit dosage amount between 0.01 and 100 mg, and said vancomycin is in a unit dosage amount between 125 and 2000 mg.

52. The method of claim 51, wherein said rifalazil is in a unit dosage amount between 1 and 50 mg, and said vancomycin is in a unit dosage amount between 500 and 2000 mg.

53. The method of claim 52, wherein said rifalazil is in a unit dosage amount between 1 and 25 mg, and said vancomycin is in a unit dosage amount between 500 and 2000 mg.

54. A pharmaceutical pack comprising (i) rifalazil in an amount effective to treat a subject having an infection of *Clostridium difficile* or prevent an infection of *Clostridium difficile* in said subject; and (ii) instructions for administering said rifalazil to said subject for treating or preventing a *Clostridium difficile* infection.

55. The pharmaceutical pack of claim 54, wherein said rifalazil is in a unit dosage amount between 0.01 and 100 mg.

56. The pharmaceutical pack of claim 55, wherein said rifalazil is in an amount between 1 and 50 mg.

57. The pharmaceutical pack of claim 56, wherein said rifalazil is in an amount between 1 and 25 mg.

58. The pharmaceutical pack of claim 57, wherein said rifalazil is in an amount between 5 and 25 mg.

59. The pharmaceutical pack of claim 54, further comprising one or more antibiotics selected from the group consisting of β -lactams, β -lactamase inhibitors, aminoglycosides, tetracyclines, lipopeptides, macrolides, ketolides, lincosamides, streptogramins, sulphonamides, oxazolidinones, quinolones, rifamycins, glycopeptides, metronidazole, garenoxacin, ramoplanin, faropenem, polymyxin, tigecycline, AZD2563, and trimethoprim.

60. The pharmaceutical pack of claim 59, wherein said β -lactam is selected from the group consisting of penicillins, cephalosporins, carbapenams, and monobactams.

61. The pharmaceutical pack of claim 59, wherein said aminoglycoside is selected from the group consisting of streptomycin, neomycin, kanamycin, paromycin, gentamicin, tobramycin, amikacin, netilmicin, spectinomycin, sisomicin, dibekalin, and isepamicin.

62. The pharmaceutical pack of claim 61, wherein said aminoglycoside is gentamicin.

63. The pharmaceutical pack of claim 59, wherein said lipopeptide is daptomycin.

64. The pharmaceutical pack of claim 59, wherein said macrolide is selected from the group consisting of erythromycin, azithromycin, and clarithromycin.

65. The pharmaceutical pack of claim 64, wherein said macrolide is azithromycin.

66. The pharmaceutical pack of claim 59, wherein said streptogramin is selected from the group consisting of quinupristin and dalbopristin.

67. The pharmaceutical pack of claim 59, wherein said oxazolidinone is linezolid.

68. The pharmaceutical pack of claim 59, wherein said quinolone is selected from the group consisting of nalidixic acid, oxolinic acid, norfloxacin, perfloxacin, enoxacin, ofloxacin, ciprofloxacin, temafloxacin, lomefloxacin, fleroxacin, grepafloxacin, sparfloxacin, trovafloxacin, clinafloxacin, gatifloxacin, moxifloxacin, gemifloxacin, and sitafloxacin.

69. The pharmaceutical pack of claim 68, wherein said quinolone is ciprofloxacin.

70. The pharmaceutical pack of claim 59, wherein said rifamycin is selected from the group consisting of rifampicin, rifabutin, rifapentine, and rifaximin.

71. The pharmaceutical pack of claim 59, wherein said glycopeptide is selected from the group consisting of vancomycin, oritavancin, dalbavancin, and teicoplanin.

72. The pharmaceutical pack of claim 71, wherein said glycopeptide is teicoplanin.

73. The pharmaceutical pack of claim 71, wherein said glycopeptide is vancomycin.

74. The pharmaceutical pack of claim 73, wherein said vancomycin is in an amount between 125 and 2000 mg.

75. The pharmaceutical pack of claim 73, wherein said vancomycin is in an amount between 500 and 2000 mg.

* * * * *

ativas entre los distintos lotes. El cumplimiento clínico; sin embargo, su incumplimiento puede dar lugar a una eficacia completa.

blecen que al menos un 75% del fármaco debe disolverse en 45 minutos. Con objeto de reducir la variabilidad interlaboratorio en los test de disolución, la USP sugiere el uso de discos estándares de ácido salicílico y prednisona como calibradores del equipo de disolución.

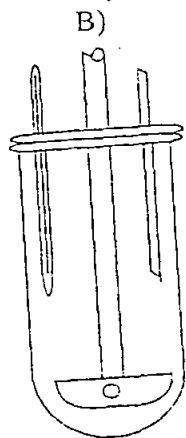
2.2.11. Comprimidos especiales

Se incluye dentro de esta categoría a aquellos comprimidos que presentan alguna característica farmacotécnica diferente de las descritas para los comprimidos convencionales. Los comprimidos especiales de mayor utilización (sublinguales y bucales, masticables, efervescentes, multicapa, vaginales, de implantación, de liberación sostenida y recubiertos) se describen a continuación.

A) Comprimidos sublinguales y bucales

Son comprimidos destinados a situarse bajo la lengua (sublinguales) o a mantenerse en la cavidad oral (bucales), donde se disuelven lentamente liberando el principio activo para producir un efecto local o ser absorbidos a través de la mucosa y ejercer un efecto sistémico. En general, presentan pequeño tamaño y una forma plana u oval. Los comprimidos bucales deben disolverse con lentitud, para lo cual en su formulación se evita la presencia de agentes disgregantes y se incorporan aglutinantes enérgicos (gelatina, goma arábiga, carboximetilcelulosa...) y lubricantes de acción hidrofóbica, del tipo del estearato magnésico, que contribuyen a aumentar el tiempo de disgregación. Además, se utilizan fuerzas elevadas de compresión para obtener comprimidos duros. Se pueden administrar de esta forma hormonas como la metiltestosterona, el citrato de oxitocina, la progesterona, antisépticos y antibióticos, etc.

Los comprimidos sublinguales suelen contener fármacos que serían destruidos o inactivados a su paso por el tracto gastrointestinal. En general, están concebidos para que el principio activo se ceda lentamente, de manera que la velocidad de liberación sea del mismo orden de magnitud que la de absorción para asegurar el máximo aprovechamiento de la dosis administrada. Si la liberación se produjera muy rápidamente, la mucosa no estaría en situación de absorber toda la cantidad de fármaco disuelta, por lo que una parte de éste sería deglutida junto con la saliva. Por el contrario, en ciertos casos especiales, cuando se recurre a esta forma de administración para el tratamiento de situaciones urgentes, como la angina de pecho o el asma, los comprimidos sublinguales deben disolverse fácilmente, permitiendo una rápida absorción del fármaco, por lo que para su obtención se emplean bajas fuerzas de compresión y se les dota de una forma lenticular y de gran superficie. De este modo se administran fármacos tales como la nitroglicerina, el clorhidrato de isoproterenol, el sulfato de isoprenalina, el dinitrato de isosórbida, la ketanserina, etc.

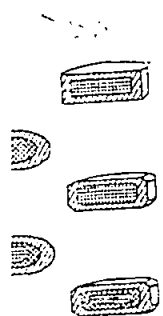


s de disolución. A) Método de cestillo. B) Método de disolución en un recipiente.

ón para llevar a cabo el ensayo, junto a la monografía de las farmacopeas, donde se debe disolver en un periodo de tiempo determinado los requerimientos de disolución de la USP.

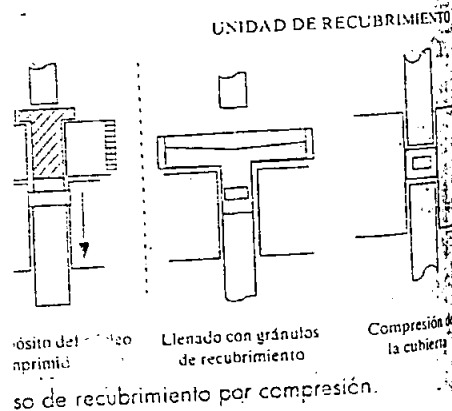
54

tintas que presenten diferentes caracteres en los tramos del tracto gastrointestinal.



dos recubiertos por compresión.

compresión constan de dos unidades de recubrimiento y el material de recubrimiento. Debe centrarse el núcleo para asegurar un recubrimiento uniforme. Un esquema del funcionamiento del núcleo y su posterior recubrimiento, cuando entipos apropiados es posible aplicar el núcleo. Por ejemplo Manesti Bicota). La película, ya que se requiere una adecuada de la cubierta y que el espesor del recubrimiento sea adecuado.



D) Recubrimiento gastrorresistente

El recubrimiento gastrorresistente o entérico, que puede ser realizado por cualquiera de las técnicas de recubrimiento descritas anteriormente, está especialmente indicado en aquellos casos en que se requiere:

- Proteger fármacos inestables en medio ácido de la acción de los fluidos gástricos (por ejemplo las enzimas y ciertos antibióticos).
- Proteger el estómago del efecto irritante de ciertos fármacos (por ejemplo el naproxeno, el diclofenac).
- Liberar preferentemente los fármacos en el intestino para conseguir una acción local (por ejemplo los antihelmínticos y los antisépticos).
- Facilitar la absorción de fármacos absorbidos preferentemente en el intestino delgado.

Los materiales de recubrimiento entérico deben garantizar la integridad de la cubierta a su paso por el estómago, manteniéndose impermeables e insolubles al pH gástrico, que oscila entre 1,5 y 4, y durante el tiempo de tránsito, que varía considerablemente de unas situaciones a otras, en especial con la presencia de alimentos (0,5-5 h, con un tiempo medio de aproximadamente 3 h).

Como materiales de recubrimiento se emplean, habitualmente, polímeros de naturaleza ácida que, en función de su pK y del pH del medio, se mantendrán no disociados (poco solubles) o se disociarán (solubles). El polímero ideal es aquel que se disuelve o se hace permeable a un pH próximo o superior a 5. Entre los más utilizados se pueden citar el acetofalato de celulosa, el acetofalato de polivinilo, los copolímeros del ácido metacrílico (Eudragits®), la goma laca, el ftalato de hidroxipropilmetilcelulosa, etc. Para conseguir un perfil de liberación adecuado se suele recurrir a la mezcla de diferentes agentes filmógenos. La figura 2.61 recoge el perfil de disolución, en función del pH, de los Eudragits L y S y de mezclas de los mismos en distintas proporciones.

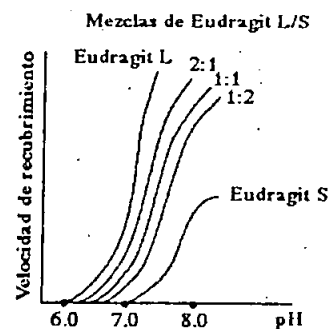


FIGURA 2.61. Perfil de disolución de diferentes mezclas de Eudragits L y S, en función del pH.