In Vitro Activities of Linezolid, Meropenem, and Quinupristin-Dalfopristin against Group C and G Streptococci, Including Vancomycin-Tolerant Isolates

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The in vitro activities of meropenem, linezolid, quinupristin-dalfopristin, vancomycin, and penicillin against 130 clinical isolates of group C and G streptococci, including vancomycin-tolerant isolates, were evaluated. Meropenem, linezolid, quinupristin-dalfopristin, vancomycin, and penicillin MICs at which 90% of the isolates were inhibited were 0.06, 2.0, 0.25, 0.5, and $\geq 0.016 \mu g/ml$, respectively. Meropenem, linezolid, quinupristin-dalfopristin, and penicillin were active against group C and G streptococci, including vancomycin-resistant strains.

There is increasing interest in the role of Lancefield group C streptococci (GCS) and group G streptococci (GGS) as emerging nosocomial and opportunistic pathogens (18, 20). The spectrum of human infection caused by these organisms includes primary and secondary bacteremia in healthy and immunocompromised hosts as well as cellulitis, endocarditis, skin and wound infections, meningitis, arthritis, osteomyelitis, pneumonia, abscesses, urosepsis, and pharyngitis (2, 4, 6, 18, 20).

The majority of GCS and GGS strains demonstrate in vitro susceptibility to penicillin, vancomycin, erythromycin, and cephalosporins (3, 17). Antimicrobial tolerance, defined as a minimum bactericidal concentration (MBC) 32 or more times greater than the MIC, among GCS and GGS has been reported for penicillin and other agents (12, 15, 16). A high rate of vancomycin tolerance among GGS isolated from patients with invasive infections was previously reported (21). We have evaluated the in vitro activities of novel agents, including linezolid and quinupristin-dalfopristin, which are active against gram-positive organisms, and meropenem, a cell wall-active carbapenem, against GGS and GCS, including vancomycin-tolerant isolates.


MATERIALS AND METHODS

A total of 130 clinical isolates of GCS and GGS (48 and 82 isolates, respectively) were obtained from patients in the United States and Finland. Isolates from Finland were collected from January 1992 through December 1995 from throat swabs and infected human material. Isolates in the United States were collected from December 1991 to March 1996 from sterile sites from patients at the Christiana Hospital in Newark, Del.

Isolate identification was performed using the API 20S Strep Strip (bioMerieux Vitek, Hazelwood, Mo.). Serotyping for GCS and GGS was performed using the PathoDx agglutination kit (Remel, Lenexa, Kans.). MICs of penicillin, vancomycin, linezolid, meropenem, and quinupristin-dalfopristin were determined using National Committee for Clinical Laboratory Standards (NCCLS) broth microdilution methods (9). Tests were performed in cation-adjusted Mueller-Hinton broth with lysed horse blood (lot 5517; Remel). Dilutions tested ranged from 16 to 0.016 $\mu g/ml$ for all drugs. Plates were prepared on-site (50 $\mu l$ per well), and antibiotic powders were supplied by the respective manufacturers. Microtiter plates were prepared to include a positive growth control well and a medium sterility well. The plates were stored at $-70^\circ$C until use, and they were thawed completely at room temperature before inoculation. Organisms were grown in 5.0 ml of Mueller-Hinton broth (Becton Dickinson, Cockeysville, Md.) in a shaker incubator for 3 h and then standardized to equal a 0.5 McFarland standard. Microtiter plates were inoculated with 50 $\mu l$ of the standardized, diluted organism suspension. Colony counts were performed on the growth control wells to determine the final inoculum count. The plates were then incubated at 35°C in 6% carbon dioxide for 20 h. Following the 20-h incubation, the plates were placed on an orbital shaker (250 rpm for 1 to 2 min) and then reincubated for an additional 4 h to equal a full 24-h incubation. The MIC was then interpreted as the lowest concentration of drug showing no visible growth in the microtiter well. The NCCLS breakpoints for streptococci were used to interpret the MICs of vancomycin (10).

Wells with no visible growth were subcultured onto blood agar plates to determine the MBC (10 $\mu l$ from each well). The blood agar plates were incubated for 24 h at 35°C in carbon dioxide. The MBC was interpreted as the lowest concentration of drug capable of killing streptococci as determined by the NCCLS M26A rejection value table (8).

All MBA assays were performed in duplicate for reliability. Broth macrodilution methods according to NCCLS standard procedures were used to confirm MIC and MBC broth microdilution results for vancomycin (8, 10).

Streptococcus pneumoniae ATCC 49619 was used for quality control for all antimicrobials and was tested with each batch of microtiter plates. The results obtained were consistently within acceptable ranges for all drugs.

RESULTS

The results of susceptibility testing are presented in Table 1. All isolates were susceptible to vancomycin; the MIC at which 50% of the isolates were inhibited (MIC50) was 0.25 $\mu g/ml$ and the MIC90 was 0.50 $\mu g/ml$. Ninety-eight isolates (75%) were found to be tolerant to vancomycin as defined by an MBC 32 times greater than the MIC. All isolates were susceptible to

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penicillin, with a MIC\textsubscript{50} and MIC\textsubscript{90} of $\leq 0.016$ µg/ml. No tolerance to penicillin was seen, with an MBC range between $0.016$ and $2.0$ µg/ml.

Linezolid showed good activity against all GGS and GCS isolates, with a MIC\textsubscript{50} and MIC\textsubscript{90} of $2.0$ µg/ml. No difference among vancomycin-tolerant strains was seen. The MBCs of linezolid were not obtained due to the bacteriostatic nature of the drug.

Meropenem was very active against all isolates, with a MIC\textsubscript{50} of $\leq 0.016$ µg/ml and a MIC\textsubscript{90} of $0.06$ µg/ml. No difference between vancomycin-tolerant and nontolerant strains was noted. The MBCs for GCS ranged from $0.25$ to $>16$ µg/ml. This range reflects one isolate for which the MBC was $>16$ µg/ml; the MBCs for all other GCS and GGS isolates were similar to the MICs.

All isolates were inhibited by quinupristin-dalfopristin at $\leq 0.016$ µg/ml. The MIC\textsubscript{50} and MIC\textsubscript{90} were identical ($0.25$ µg/ml) for vancomycin-tolerant and nontolerant strains. No tolerance to quinupristin-dalfopristin was seen, with MBCs similar to MICs for all isolates. All MIC and MBC results obtained by broth macrodilution methods were nearly identical to the broth microdilution results presented in Table 1.

**DISCUSSION**

The purpose of this study was to characterize the antibiotic susceptibility patterns of GCS and GGS. The strains utilized included vancomycin-tolerant human isolates. There are few reported data on the activity of these agents against GCS and GGS (11).

Linezolid is the first of a new class of synthetic antimicrobial agents, the oxazolidinones, with potent activity against gram-positive bacteria. This novel antimicrobial agent has a unique method of bacterial protein synthesis inhibition. Linezolid binds to a site on the bacterial 23S rRNA of the 50S subunit and prevents the formation of a functional 70S initiation complex, which is an essential component of the translation process. This unique mechanism precludes cross-resistance with other existing antimicrobial agents (7, 19, 22).

Meropenem is a cell wall-active parenteral carbapenem antimicrobial with an extended spectrum of in vivo activity against gram-positive and gram-negative organisms, and it is relatively resistant to hydrolysis by beta-lactamases, including extended-spectrum beta-lactamases (14). One isolate of GCS exhibited tolerance to meropenem. To our knowledge, this is the first reported meropenem-tolerant streptococcal isolate. Antimicrobial tolerance is usually associated with cell wall-active agents, and meropenem can be linked possibly to its mechanism of action.

Quinupristin-dalfopristin is a recently approved combination of the semisynthetic parenteral streptogramins quinupristin (30%) and dalfopristin (70%). Its in vitro spectrum of activity includes most multiresistant gram-positive aerobes. Streptogramins exert their activity by inhibiting protein synthesis. Individually, the two agents are bacteriostatic, but in combination, they are bactericidal against streptococci (5).

In our study, linezolid, meropenem, and quinupristin-dalfopristin all showed excellent activity against all our GCS and GGS isolates, including vancomycin-tolerant isolates.

The significance of in vitro vancomycin tolerance is uncertain. Recent evidence obtained by Novak et al. (13) demonstrated a molecular mechanism for vancomycin in *S. pneumoniae*. A rabbit meningitis model utilized in their studies indicated the failure of vancomycin therapy to eradicate tolerant organisms from the cerebrospinal fluid. Concerns about potential antimicrobial tolerance in GCS and GGS and reports of clinical failures in patients with severe infections has led many authors to recommend combination therapy for synergy (aminoglycoside plus a cell wall-active agent) in the treatment of these patients (1, 16, 18, 20).

In summary, our in vitro data suggest that linezolid, quinupristin-dalfopristin, and meropenem have excellent activity against clinically significant isolates of GCS and GGS, including those previously found to be tolerant to vancomycin. These newer agents may prove valuable as monotherapy or in combination with other agents in the treatment of high-risk patients with invasive GCS and GGS infections who cannot be treated with penicillin, which remains the drug of choice.

**REFERENCES**

of the Committee on Infectious Diseases, 24th ed. American Academy of Pediatrics, Elk Grove Village, Ill.


