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THE CHALLENGE OF MULTIDRUG RESISTANCE: THE TREATMENT OF GRAM-NEGATIVE ROD INFECTIONS

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ABSTRACT—Infections caused by multidrug-resistant gram-negative bacteria are an increasing problem worldwide. Treatment of these microorganisms is a challenge because resistance limits dramatically therapeutic options. In this review, we discuss data of *in vitro* susceptibility and clinical studies of possible agents for the management of these infections. Currently, published data are limited, and there are no randomized clinical trials involving the treatment of infections caused by multidrug–resistant gram-negative rods. For imipenem-resistant *Acinetobacter* spp., most studied options are polymyxins and sulbactam. No newer antimicrobials active against *Pseudomonas aeruginosa* are available or under investigation. Tigecycline presents a broad spectrum of activity *in vitro* but has been studied mainly as treatment of community-acquired infections, as has ertapenem. They are potential options against extended-spectrum β-lactamase–producing Enterobacteriaceae, and tigecycline may be useful in treating *Acinetobacter* infections.

KEYWORDS—Polymyxins, sulbactam, tigecycline, ertapenem, *Pseudomonas aeruginosa*, *Acinetobacter* spp., extended-spectrum β-lactamase–producing Enterobacteriaceae

INTRODUCTION

There are a number of gram-negative rods that cause multidrug-resistant infections. In 2004, in the hospital of the University of São Paulo, Brazil, resistance was 26% to imipenem of blood isolates of *Acinetobacter* spp. and greater than 70% to *Pseudomonas aeruginosa* in intensive care units. Among *Klebsiella pneumoniae* bloodstream infections, resistance was 80% to cephalosporins and greater than 60% to ciprofloxacin (personal communication). In multicenter worldwide studies, the prevalence of resistance is high (1), with 13% and 18% resistance to imipenem of *Acinetobacter* spp. and *P. aeruginosa*, respectively. In Brazil, the SENTRY Study demonstrated 30% and 22% resistance to imipenem of *P. aeruginosa* and *Acinetobacter* spp., respectively (2). One study reported that 67.7%, 62.9%, and 29% of *Acinetobacter baumannii*, *P. aeruginosa*, and *K. pneumoniae* isolates from all clinical specimens from intensive care unit patients, respectively, were resistant to imipenem (3).

The treatment of these microorganisms is a challenge. The first question is which drugs to use. There are few newly commercialized drugs such as ertapenem and tigecycline that present activity against gram-negative rods, but at the moment, old drugs such as polymyxins are used, as well as sulbactam, a β-lactamase inhibitor with intrinsic activity against *Acinetobacter*.

IN VITRO SUSCEPTIBILITY

Polymyxins

These are peptide drugs developed in the 1940s that were abandoned in the 1980s due to the development of less toxic options to treat gram-negative bacilli. There are two polymyxins in clinical use: polymyxin B and colistin. The spectrum of activity of polymyxins involves a large number of gram-negative bacilli: *Escherichia coli*, *K. pneumoniae*, *Enterobacter* spp., *Acinetobacter* spp., and *P. aeruginosa*. Activity is not good against *Providencia* spp., *Serratia marcescens*, *Proteus mirabilis*, and *Proteus vulgaris*. Activity against *Burkholderia cepacia* is controversial (4).

Susceptibility testing for polymyxins is a problem. The break points are not absolutely established. The Clinical Laboratory Standards Institute (5) recommends the following break points for polymyxin B: susceptible, less than or equal to 2 μg/mL; intermediate, 4 μg/mL; and resistant, greater than or equal to 8 μg/mL for *P. aeruginosa* and susceptible, less than or equal to 2 μg/mL and resistant, greater than or equal to 4 μg/mL for *Acinetobacter* spp. Interpretative criteria by disk-diffusion are established for *P. aeruginosa*: susceptible, greater than or equal to 12 mm and resistant, less than or equal to 11 mm. However, it is not clear whether these break points are clinically relevant. In addition, most clinical laboratories do not use the determination of minimum inhibitory concentration (MIC) routinely. The studies on the performance of disk-diffusion method showed disappointing results. Gales et al. (6), evaluating 200 blood isolates of *Acinetobacter* spp., *B. cepacia*, and other gram-negative bacilli, showed that very major errors occurred in 5% for colistin and 6% for polymyxin B. Different diameters were evaluated, but no adequate break points for disk-diffusion were found. However, these authors determined that resistance for polymyxin B determined by MIC was predictive for colistin. In another study, 78 *P. aeruginosa* strains were tested by broth microdilution, disk-diffusion, and E-test. Major and very major errors were observed in 1.2% and 11.5% comparing polymyxin disk-diffusion with the reference method broth microdilution, respectively (7).

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Sulbactam

Although this drug was originally developed to be used as a β-lactamase inhibitor, it presents *in vitro* activity against *Acinetobacter* spp. (8) and has been used to treat infections caused by this organism.

In vitro susceptibility of *Acinetobacter* to sulbactam is difficult to determine. Automated methods usually have fixed concentrations of the drug because it is evaluated as a β-lactamase inhibitor, and only the concentrations of the associated drug (such as ampicillin) vary. The break points used to interpret MIC results are not well studied and may not predict clinical outcomes. The Clinical Laboratory Standards Institute (5) has defined the following break points for ampicillin-sulbactam (A-S): susceptible, less than or equal to 8/4 μg/mL and resistant, greater than or equal to 32/16 μg/mL. A study involving 221 isolates of *Acinetobacter calcoaceticus–baumannii* complex found MIC_{50} to be 8 μg/mL and MIC_{90} to be 16 μg/mL for sulbactam alone (9).

The disk-diffusion method also presented problems. One study reported 9.8% very major errors when comparing disk-diffusion diameter and MIC determination for A-S (10). The disk diffusion break points used were susceptible, greater than 13 mm; resistant less than 12 mm; and intermediate, 12 to 13 mm.

Ertapenem

The spectrum of activity involves gram-negative rods, but the activity against *P. aeruginosa* and *Acinetobacter* spp. is poor. Against extended-spectrum β-lactamase (ESBL)–producing organisms such as *E. coli* and *K. pneumoniae*, *in vitro* activity is good (11). *In vitro* studies have shown susceptibility of 95% to 100% of these organisms to ertapenem (12).

Tigecycline

The spectrum of activity of this drug is very ample. When evaluating only gram-negative rods, activity was observed against Enterobacteriaceae with MIC_{50} of 0.25 μg/mL and MIC_{90} of 1 μg/mL. Susceptibility was 96.7% considering a break point of less than or equal to 2 μg/mL as susceptible. There were no significant geographical differences. Activity against ESBL producers is also good. Activity against *A. baumannii* has been described, with MIC_{50} and MIC_{90} of 0.5 and 1 μg/mL, respectively. However, the problem is the lack of defined break points for this organism. There is no significant activity against *P. aeruginosa* (13).

CLINICAL EFFICACY

Polymyxins

Polymyxins seem to have a surface detergent effect, disrupting calcium and magnesium bridges of the phospholipid layers of the outer membrane, disrupting the membrane itself (4). The main concern with this class of drugs is toxicity, mainly nephrotoxicity and neurotoxicity. In a recent review of old and recent studies, nephrotoxicity was higher in older studies. In the more recent studies, among patients without cystic fibrosis, it varied from none at all to 37% in patients with normal baseline function (14). Paresthesias were reported to occur in up to 27% of patients with intravenous polymyxins. In recent studies, however, severe neurotoxicity such as neuromuscular blockade or apnea has not been reported (14), maybe due to the patients' condition, frequently under mechanical ventilation.

There are no randomized clinical trials with polymyxins. Currently, only a few comparative studies are published, and the most recent clinical experience is with colistin.

A case series with 60 infections caused by multidrug–resistant *P. aeruginosa* and *Acinetobacter* spp. treated with intravenous colistin demonstrated an overall clinical success rate of 58%. However, among cases of pneumonia, success occurred in only 25% of 20 infections. Success for infections at other sites varied: 78% for primary bloodstream, 83% for urinary tract, and 80% for central nervous system infections (15). Since then, there have been a few reports of treatment of pneumonia with success rates superior to 50%, most with less than 20 cases (16, 17). However, a study involving 61 cases of pneumonia caused by *P. aeruginosa* or *A. baumannii* yielded a success rate of 73.8% (18).

One retrospective study of ventilator-associated pneumonia (VAP) caused by *Acinetobacter* spp. compared 21 carbapenem-resistant cases treated with colistin and 14 carbapenem-susceptible cases treated with imipenem. The success rate for either group was 57% (16). Surprisingly, kidney failure was more common in the imipenem group (42% vs. 24%), although this difference was not statistically significant. In this study, in-hospital mortality was 62% and 65% for colistin and imipenem, respectively.

Another comparative study involved 185 cases of infection caused by *Acinetobacter* or *P. aeruginosa* and was prospective but not randomized. They compared 55 infections treated with colistin and 130 treated with other drugs (81% of these were treated with a carbapenem). On day 6 of treatment, 15% and 17% of patients were considered clinically cured in the colistin and noncolistin groups, respectively (19).

Recently, there has been some experience with the use of inhaled polymyxin in noncystic fibrosis patients. Experience has been published as case series mainly with colistin (18) and one report of salvage therapy with inhaled polymyxin B (20).

Intraventricular and intrathecal use of polymyxins has been reported in case reports or small case series. Most of the patients also received systemic antibiotics. Results were, in general, good, with success rates greater than 80% (21, 22). However, gram-negative meningitis or ventriculitis has been reported to respond to intravenous colistin treatment alone (15).

Sulbactam alone or in combination

Sulbactam seems to be the active drug in the combination against *Acinetobacter* spp. In a series of cases of nonsevere infections, success occurred in 17 of 18 patients treated with sulbactam alone (23). This study also demonstrated success in 22 of 23 cases treated with the combination A-S.

A case series involving 40 severe infections yielded a clinical success rate of 67.5% (24). Randomized clinical trials are not available, and comparative studies are rare. Three retrospective studies comparing A-S with imipenem were published. One involved 50 cases of bacteremia, of which 8

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were treated with A-S. Success rate was 83% for A-S and 88% for imipenem (25). In another study with 48 cases of *Acinetobacter*, the success rate with imipenem was 56% in 18 patients compared with 84% in 30 patients treated with A-S. Aminoglycosides were associated in 44% of cases with imipenem and 37% of those with A-S (26). The third study involved 77 patients with VAP, of which 14 used A-S. Success rates were 93% with A-S and 83% with imipenem. In the A-S group, 12 of 14 isolates were resistant to imipenem. Aminoglycosides were more frequent in the A-S group (27).

Finally, we performed a retrospective study comparing the treatment of 82 severe infections caused by *Acinetobacter* spp. treated with colistin or polymyxin B, with a group of 85 infections treated with A-S. Clinical success rates were 39% for polymyxin and 59% for A-S; mortality rates were 50% and 33% at the end of treatment and 77% and 63% while in hospital for polymyxin and A-S, respectively. In a multivariate analysis, treatment with A-S was statistically superior to polymyxin when evaluating clinical outcome and mortality during treatment (28).

The treatment of central nervous system *Acinetobacter* infections is a concern. Cure in treating *Acinetobacter* meningitis was reported in six of eight patients (29); however, clinical failure has been reported (24), and penetration in the CNS has been described as poor (30).

Tigecycline

Tigecycline is the first drug available for use of the new glycylcycline class (31). As previously described, its spectrum is ample and involves susceptibility to *Acinetobacter* spp. and ESBL-producing Enterobacteriaceae.

However, randomized clinical studies have not been done with infections caused by these agents. Randomized controlled studies are available for community-acquired skin and skin-structure and intra-abdominal infections (31) in which multidrug-resistant agents are not important. There is a case series of *A. baumannii* infections treated with tigecycline (32). Most were cases of VAP, and only five patients received tigecycline alone. Most patients received a combination of tigecycline with imipenem or colistin. There were only four cases of failure, all in VAP. In one of them, resistance to tigecycline developed during treatment (MIC, 12 μg/mL). Resistance of *Acinetobacter* to tigecycline has been a concern due to another report of two clinically relevant blood isolates with high MICs (4 and 16 μg/mL) in patients using the drug (33).

Ertapenem

Ertapenem, although developed after the other carbapenems, has a narrower spectrum of activity. However, it presents good *in vitro* activity against ESBL-producing Enterobacteriaceae as previously presented, although imipenem and meropenem are considered the preferred options. The randomized controlled trials with ertapenem included community-acquired infections such as intra-abdominal, pelvic, skin and skin structure, pneumonia, urinary tract, and diabetic foot infections (34). However, there are two case series that involved mostly *K. pneumoniae* and *E coli*, but *Enterobacter cloacae*, *P. mirabilis*, and *Citrobacter freundii* also occurred (35, 36). Of 20 patients with VAP, 16 presented clinical success (35). Among less severe infections, success was 94% of 39 infections that responded clinically; however, 15% presented a relapse (36). All relapses occurred in patients with urinary tract infections with indwelling urinary catheters.

In conclusion, there are no randomized clinical trials involving the treatment of infections caused by multidrug–resistant gram-negative rods. Polymyxins are an option to treat a broad range of microorganisms, and there are case series and nonrandomized comparative studies involving infections caused by *P. aeruginosa* and *Acinetobacter* spp. The main problem with this class of drugs is its nephrotoxicity, which varied greatly among studies. Sulbactam used alone or in combination with ampicillin is a possible alternative to treat infections caused by *Acinetobacter* spp. because case series and comparative studies have been published. Tigecycline presents a broad spectrum of activity *in vitro* but has been studied mainly as treatment of community-acquired infections, as has ertapenem. They are potential options against ESBL-producing Enterobacteriaceae, and tigecycline may be useful to treat *Acinetobacter* infections. However, to date, there is very limited experience with these indications, and caution is recommended. Neither drug is active against *P. aeruginosa*.

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