DETECTION OF TICARCILLIN-CLAVULONIC ACID SUSCEPTIBILITY WITH MICRODILUTION METHOD IN *CITROBACTER*, *HAFNIA*, *PROTEUS* AND SOME GRAM NEGATIVE BACTERIA

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SUMMARY

The broth dilution method has been regarded as a good alternative test for detection of susceptibilities to antimicrobial agents. In this study, the antimicrobial activity of ticarcillin-clavulonic acid (TIM) was investigated by the minimal inhibitory concentration (MIC) method on strains of *Aeromonas*, *Citrobacter*, *Hafnia*, *Morganella*, *Proteus*, *Pseudomonas* and Gram negative bacteria isolated from raw milk. The isolate collection included 91 Gram negative strains. Fifty-one (56.04%) isolates were found sensitive (MIC ≤8 µg/ml), 12 (13.19%) isolates were found intermediately sensitive (MIC 16-32 µg/ml), and 28 (30.77%) isolates were found resistant (MIC ≥64 µg/ml) to TIM.

KEY WORDS

Aeromonas, Citrobacter, Hafnia, Morganella, Proteus, Pseudomonas, antibiotic susceptibility test, microdilution method, ticarcillin-clavulonic acid, minimal inhibitory concentration

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INTRODUCTION

The incidence of serious microbial infections with Gram negative bacteria continues to increase. Until recent years ticarcillin-clavulonic acid (TIM) was the drug available for therapy in patients with microbial infections. There have many reports documenting such combination therapy of certain drugs. The antibiotic resistance of 42 clinical isolates of *Escherichia coli*, *Pseudomonas aeruginosa*, *Enterobacter cloacae*, *Klebsiella pneumoniae,* and *Serratia marcescens* were examined by the microdilution method /1/. All strains were susceptible to TIM in this study.

In the present study, we examined the *in vitro* activity of TIM against 91 Gram negative isolates isolated from raw milk using the microdilution method in order to develop improved clinical therapies.

MATERIALS AND METHODS

Test organisms

Bacterial strains were isolated from raw milk. Four hundred samples of raw milk were obtained from a factory in which milk was collected from different dairy farms and stored in cooler tanks.

The isolates were identified with standard methods and stored at -20°C in peptone water containing 10% glycerine until used in the study. Prior to use, microorganisms were thawed, and subcultured at least twice on brain heart infusion (BHI) agar plates. Quality control was performed by testing the following strains according to the recommendations of the National Committee for Clinical Laboratory Standards (NCCLS) /2/. Ninety-one Gram negative isolates were tested for their susceptibilities to TIM by the broth dilution method to determine the minimal inhibitory concentration (MIC).

Antimicrobial drug

TIM was obtained from the manufacturer as a powder and a 1,280 µg/ml stock solution of TIM was prepared by dissolving with dimethylsulfoxide and fluconazole in sterile water and stored at -70°C until use. The final drug concentrations ranged from 128 to 0.125 µg/ml TIM, obtained by 10 two-fold serial dilutions.

MICs were determined by the microdilution method based on the standard method for antimicrobial susceptibility testing proposed by the NCCLS with peptone water medium /2/. Sterile flat-well microtiter plates were used.

Microorganisms were grown on BHI agar medium at 37°C for 24 h. The wells were inoculated with 100 µl of the culture suspension diluted to a final inoculum of 2.5×10^3 cells/ml with peptone water medium. The MIC of TIM was the lowest drug concentration that resulted in a visible turbidity <80% inhibition compared with the control microorganism growth. The effect of TIM was determined by checkerboard titration and expressed as MICs /2/.

RESULTS

Those isolates found sensitive to TIM and their MIC ranges are summarized in Table 1. The other isolates: *Citrobacter diversus* (1), *Enterobacter nimipressuralis* (1), *Erwinia chrysanthemi* (1), *Escherichia coli* (1), *Klebsiella terrigena* (1), *Moraxella* spp. (1), *Providencia stuarti* (1), *Serratia liquefaciens* (1), *and Yersinia pestis* (1), were found resistant to TIM except *Enterobacter nimipressuralis*. The MICs of TIM ranged between 0.25 and 128 µg/ml in this study.

DISCUSSION

The results of our study show that TIM was highly active (0.5-1 µg/ml) aganist *Aeromonas hydrophila*. However, TIM was least active (128 µg/ml) against *Proteus penneri and Morganella morgani* in comparison with other Gram negative isolates in this study. Similar findings were reported previously /3/.

A plasmid vector containing a TIM resistance gene has been found in strains of *Pseudomonas aeruginosa* and *Escherichia coli* /1/. These observations are in agreement with our findings.

Among the various pathogens responsible for urinary infections is *Morganella morganii*. The different strains of this species are usually resistant to ampicillin, to the amoxicillin-clavulanic acid combination, and to cephalothin, and they are usually susceptible to other antibiotics active against Gram-negative bacilli /4/. A strain of *M. morganii*, showing resistance to ceftazidime and aztreonam and reduced

TABLE 1

Gram negative isolates which were sensitive to TIM and their MIC ranges

Isolates (n)	No. of sensitive strains	MIC range (μg/ml)
Aeromonas hydrophila (1)	1	0.25
Aeromonas salmonicida (1)	1	4
Cedecea neteri (1)	1	0.5
Citrobacter amalonaticus (2)	1	8-16
Citrobacter braakii (1)	1	2
Citrobacter freundii (11)	7	1-64
Eikenella corrodens (2)	1	1-16
Enterobacter cancergenus (1)	1	4
Enterobacter cloacae (3)	2	1-64
Hafnia alvei (5)	1	8-64
Morganella morgani (8)	3	0.5-128
Pasteurella multocida (1)	1	4
Proteus mirabilis (13)	8	0.25-64
Proteus penneri (6)	1	8-128
Proteus vulgaris (6)	3	2-64
Providencia alcalifaciens (1)	1	4
Providencia rettgeri (6)	5	1-64
Pseudomonas fluorescens (7)	6	0.25-64
Pseudomonas maltophilia (1)	1	1
Pseudomonas putida (1)	1	2
Salmonella choleraesuis (2)	2	0.5-2
Serratia grimesii (1)	1	0.5
Serratia marcescens (1)	1	8

susceptibility to cefotaxime by the disk diffusion method, was isolated from the urine of a hospitalized neonate. The double disk test for synergy between expanded-spectrum cephalosporins and clavulanic acid was performed, and the positive result indicated the presence of an extended spectrum beta-lactamase (ESBL) producer. This isolate was also resistant to netilmicin, gentamicin, and amoxicillin. The MICs for the *M. morganii* strain and its transconjugant were determined by the E test (AB Biodisk). The values obtained showed that the *E. coli* transconjugant was more resistant to aztreonam than *M. morganii* /5/.

A clinical isolate of *Pseudomonas aeruginosa* showed resistance both to extended-spectrum cephalosporins and to aztreonam. A typical double-disk synergy image was detected when ceftazidime or aztreonam was placed next to a clavulanic acid disk on an agar plate. Additionally, MICs were determined for reference strain *P. aeruginosa* and its *in vitro*-obtained, stably de-repressed, cephalosporinase-producing mutant. This mutant, obtained after selection on ceftazidime-containing plates, produced an 85-fold increase of cephalosporinase activity determined as described for an *Enterobacter cloacae* isolate. The MIC of ceftazidime (32 μg/ml) was reduced to 8 μg/ml in the presence of clavulanic acid /6/.

In one study, *E. coli* showed decreased susceptibility to all of the β-lactams tested except cefoxitin and imipenem; the MICs of the extended-spectrum cephalosporins and aztreonam were markedly reduced in the presence of clavulanic acid, indicating the presence of an ESBL /7/.

Enterobacter cloacae was isolated with *Escherichia coli* from the urine of a patient living in a nursing home. Both isolates were resistant to ticarcillin (MICs, 1,024 μg/ml), without significant potentiation of its activity by 2 μg of clavulanate per ml (MIC, 512 μg/ml), and susceptible to naturally active cephalosporins. This inhibitor-resistant phenotype was conferred in both strains by similar conjugative plasmids of 40 kb and 30 kb which also conveyed resistance to sulfonamides and trimethoprim /8/.

In other studies, *P. aeruginosa*, *P. cepacia*, and *P. maltophilia* were tested by the MIC method. These isolates were highly sensitive to TIM /9/. Some clinical isolates (18 *E. coli*, 6 *K. Pneumonie*, 5 *Proteus* spp. and 5 *P. aeruginosa*) were sensitive to TIM /10/.

In one study, *Serratia marcescens* KU3838 was isolated from the urine of patient with a urinary tract infection and was found to contain the metallo-β-lactamase gene. This strain were resistant to carbapenems and various antimicrobial agents/11/.

In the present study, susceptibility testing of *Aeromonas, Citrobacter, Hafnia, Morganella, Proteus, Pseudomonas* and some Gram negative isolates was performed by the broth dilution method. Among these isolates, 51 (56.04%) were found sensitive (MIC ≤8 μg/ml), 12 (13.19%) isolates were found intermediately sensitive (MIC 16-32 μg/ml), and 28 (30.77%) isolates were found resistant (MIC ≥64 μg/ml) to TIM.

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