



Original Research Article

Invitro susceptibility of gram negative bacilli to the newer anti-microbial agents

Tariq Ahmad Lone^{1*}, E.M.Mohamed Younis¹ and Reyaz Ahmad Lone²

¹Department of Microbiology Sree Amman Arts and Science College,
Erode-638102, Tamil Nadu India

²Department of Biotechnology, Periyar University Salem-636011, Tamil Nadu, India

*Corresponding author

A B S T R A C T

Keywords

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205 clinical isolates of gram negative bacteria were characterized based on morphological and biochemical reaction which revealed the presence and confirmation of 105-*Escherichia coli*, 41-*Klebsiella pneumoniae*, 40-*Pseudomonas aeruginosa*, 14-*Acinetobacter baumannii* and 5-*Proteus vulgaris*. The antibiotic sensitivity test presented in the Table 2 revealed that Tigecycline (Tige-10 μ g) exhibited maximum 100% susceptibility and Ceftazidime (Caz-30 μ g) exhibited minimum 38% sensitivity against the *Escherichia coli* isolates trains. In case of *Klebsiella pneumoniae* isolates showed 100% susceptibility against Tigecycline (Tige-10 μ g) and 53% susceptibility against Tobramycin (Tob-10 μ g). While in *Pseudomonas aeruginosa* isolates Tigecycline (Tige-10 μ g) showed 97% susceptibility and minimum 30% susceptibility against Ceftazidime (Caz-30 μ g). In case of *Acinetobacter baumannii* isolates Tigecycline (Tige-10 μ g) revealed 100% susceptibility and Netilmicin (Net-30 μ g) had shown 35% susceptibility. While in case *Proteus vulgaris* isolates Tigecycline (Tige-10 μ g) revealed 100% susceptibility and Tobramycin (Tob-10 μ g) had shown 10% susceptibility.

Introduction

Numerous classes of antimicrobials are currently available for physicians to use in the treatment of patients with infections; however, the pace of antibiotic drug development has slowed during the last decade (Kumarasamy et al., 2010). New antibiotics that have been discovered and introduced into clinical practice in the last few years are active mostly against Gram-positive organisms, whereas when

targeting resistant Gram-negative bacteria, clinicians are forced to rediscover old drugs, such as polymyxins and fosfomycin (Lye et al., 2008). Among new antibacterial active against Gram-negative microorganisms that are already on the market, tigecycline, the first Food and Drug Administration (FDA)-approved representative of the glycylicyclines, and doripenem, a new carbapenem, seem the

most promising (Kelesidis et al., 2008). In *Enterobacteriaceae*, the main resistance problems stem from production of ESBL, inducible chromosomal cephalosporinases and carbapenemases, including *Klebsiella pneumoniae* carbapenemase (KPC)-hydrolyzing β -lactamases (Falagas et al., 2009). Infections due to ESBL-producing *Escherichia coli* and *Klebsiella* spp. continue to increase in frequency and severity. In an interesting meta-analysis of 16 studies, bacteremias caused by ESBL-producing pathogens were significantly associated with delayed initiation of effective therapy and increased crude mortality (Rodriguez-Bano et al., 2008). Moreover, carbapenem resistant *Enterobacteriaceae* are increasingly recognized as the cause of sporadic infections and outbreaks worldwide (Gavin et al., 2006). Aggressive infection-control practices are required to abort epidemic outbreaks. Rates of infection by resistant *Pseudomonas aeruginosa* continue to increase in the United States and globally, as does resistance to β -lactams, quinolones, aminoglycosides and carbapenems (Henwood et al., 2002).

Materials and Methods

Setting and samples

205 clinical isolates of gram negative bacteria were isolated from the various source samples such as Urine, Sputum, Pus, Vaginal swabs, Blood, Bronchial wash and other body fluids from the Govt Hospital Erode District of Tamil Nadu India and brought to the laboratory under aseptic conditions. The samples isolates were processed for the identification based on cultural characteristics and reactions in standard biochemical tests (Holt et al., 1994).

Antibiotic susceptibility testing

The susceptibility of the isolates was determined against antibacterial agents by disc diffusion method (Insa et al., 2007). They included Ceftazidime (Caz-30 μ g), Gentamicin (G-10 μ g), Tobramycin (Tob-10 μ g), Amikacin (Ak-30 μ g), Netilmicin (Net-30 μ g), Imipenem (Ipm-10 μ g), Meropenem (Mem-10 μ g), Tetracycline (Te-30 μ g) and newer drugs tested include Polymyxin (Poly-10 μ g) as well as Tigecycline (Tige-10 μ g). The susceptibility and resistance was determined based on the interpretative criteria recommended by the National Committee for Clinical Laboratory Standards (NCCLS). *Escherichia coli* ATCC 25922 was used as the quality control strain.

Disc Diffusion method (Kirby-Bauer method)

At least three to five well-isolated colonies of the same morphological type are selected from an agar plate culture (Insa et al., 2007). The top of each colony is touched with a loop, and the growth is transferred into a tube containing 5ml of a suitable broth medium, such as tryptic soy broth. The broth culture is incubated at 35°C until it achieves or exceeds the turbidity of the 0.5 McFarland standards (usually 2 to 6 hours). The turbidity of the actively growing broth culture is adjusted with sterile saline or broth to obtain turbidity optically comparable to that of the 0.5 McFarland standards. This results in a suspension containing approximately 1 to 2 x 10⁸ CFU/ml for *Escherichia coli* ATCC 25922. To perform this step properly, photometric device was be used to set adequate light needed to visually compare the inoculum tube and the 0.5 McFarland standard against a card with a white

background and contrasting black lines. An optimally, within 15 minutes after adjusting the turbidity of the inoculum suspension, a sterile cotton swab is dipped into the adjusted suspension. The swab should be rotated several times and pressed firmly on the inside wall of the tube above the fluid level. This will remove excess inoculum from the swab. The dried surface of a Mueller-Hinton agar plate is inoculated by streaking the swab over the entire sterile agar surface. This procedure is repeated by streaking two more times, rotating the plate approximately 60° each time to ensure an even distribution of inoculum. As a final step, the rim of the agar is swabbed.

Double disc-diffusion (DDD) method For detection of ESBL

The DDD method that was used here employed ceftazidime (30 mg), and with a combination of the same antibiotics with the addition of clavulanic acid ((Insa et al., 2007). A broth culture of the test organism was adjusted to a 0.5 McFarland standard and inoculated onto Mueller-Hinton agar (Oxoid). The combination discs and the corresponding standard cephalosporin disc were placed at the recommended distance from each other on the plate. The plates were incubated at 37°C for 24 hours aerobically before the zone sizes were recorded. A positive result was indicated by a zone-size difference of 5 mm diameter between the combination disc and the corresponding standard antibiotics.

Results and Discussion

Identification of Isolates

205 clinical isolates of gram negative bacteria were characterized based on morphological and biochemical reactions

revealed the presence and confirmation of 105-*Escherichia coli*, 41-*Klebsiella pneumoniae*, 40-*Pseudomonas aeruginosa*, 14-*Acinetobacter baumannii* and 5-*Proteus vulgaris* table 1.

Antibiotic susceptibility

The antibiotic sensitivity test presented in the Table 2 revealed that Tigecycline (Tige-10µg) exhibited maximum 100% susceptibility and Ceftazidime (Caz-30µg) exhibited minimum 38% sensitivity against *Escherichia coli* isolates strains. In case of *Klebsiella pneumoniae* isolates showed 100% susceptibility against Tigecycline (Tige-10µg) and 53% susceptibility against Tobramycin (Tob-10µg). While in *Pseudomonas aeruginosa* isolates Tigecycline (Tige-10µg) showed 97% susceptibility and minimum 30% susceptibility against Ceftazidime (Caz-30µg). In case of *Acinetobacter baumannii* isolates Tigecycline (Tige-10µg) revealed 100% susceptibility and Netilmicin (Net-30µg) had shown 35% susceptibility. While in case *Proteus vulgaris* isolates Tigecycline (Tige-10µg) revealed 100% susceptibility and Tobramycin (Tob-10µg) had shown 10% susceptibility.

Bacterial resistance to the commonly used anti-microbial agents is increasing and it is a matter of concern, particularly in patients with serious and complicated nosocomial infections. Emergence and spread of ESBL production in Enterobacteriaceae and carbapenem resistance among Gram negative bacteria have led to the limited therapeutic options, resulting in increased morbidity and mortality (Rodriguez-Bano et al., 2006 ; Parchuri et al., 2005). The development of new antimicrobial agents with novel modes of action is critically needed to keep in pace with the development and spread of drug resistance

mechanisms among bacteria (Zanetti et al., 2003). Meropenem and imipenem are routinely used as therapy for *P. aeruginosa* and *A. baumannii* infections. Between the two carbapenems, meropenem is noted to be more potent against *P. aeruginosa* and Imipenem is more potent against *Acinetobacter* sp (Freire et al., 2010). Tigecycline was highly active against the ESBL producing Enterobacteriaceae (Thomson and Moland, 2001). Although carbapenems are widely regarded as the drugs of choice for treatment of infections caused by ESBL producing organisms, production of beta-lactamases capable of hydrolyzing carbapenems have been reported from Enterobacteriaceae (Poirel and Nordmann, 2006). The previous study conducted on tigecycline against gram positive and gram negative isolates in a Tertiary Care Hospital in India shows tigecycline is a potent antimicrobial agent against MRSA, ESBL producing Enterobacteriaceae and multi-drug resistant *Acinetobacter baumannii* and 70.6% of MDR *Acinetobacter* species were susceptible to tigecycline (Tripodi et al., 2007; Anthony et al., 2008). Hence the use of tigecycline should be strictly monitored to prevent the development and dissemination of resistance against tigecycline, which is the last resort in the treatment of MDR *Acinetobacter baumannii* infections (Samra et al., 2007). A study on antibiotic Core resistance in extended spectrum β lactamase Producing Enterobacteriaceae and invitro activity of tigecycline in Spain reported, Tigecycline is not affected in multi resistant ESBL-producing Enterobacteriaceae (Morosini et al., 2006). Significantly tigecycline has recently been advocated as one of the few options with in vitro activity against certain Metallo β lactamase-producing gram-negative pathogens (Montero et al.,

2004) and particularly in the case of carbapenemase-producing *K. pneumoniae* isolates (Song et al., 2007). However tigecycline has to cope with intrinsic resistance in *Proteus* spp and an overall decreased activity in *Serratia* spp and some *K. pneumoniae* strains and *Enterobacter* spp (Segal-Maurer et al., 1999). In their study a tigecycline MIC₉₀ of 1 μ g/ml was observed and no fully resistant isolates were observed. Study conducted in infectious disease and clinical microbiology, Rabin medical centre (Bielinson campus), Petah Tiqva , Israel, shows the prevalence of ESBL producing organisms was significantly higher among *K. pneumoniae* than *E. coli* isolates, All these isolates were sensitive to carbapenams, polymyxin and Tigecycline (Bishara et al., 2005). In our research work a total of 205 isolates were studied which included *E. coli* (51%), *Klebsiella* sp (20%), *Pseudomonas* spp (19%), *Acinetobacter* spp (6%), and *Proteus* spp (2%).The isolates showed 100% sensitivity to Tigecycline followed by 97% to polymyxin, Imipenem and Tetracycline (Castanheira et al., 2008; Bratu et al., 2005). Ceftazidime resistance is a major problem for the treatment of serious infections caused by drug resistant bacteria and overall resistance was more for Merpoenem than for Imipenem (Hirsch et al., 2010). Polymyxin B can be a useful alternative for drug resistant bacteria, especially for MDR *Pseudomonas* spp (Mushtaq et al., 2004; Bellais et al., 2002). Tigecycline showed 100% sensitivity against the isolates tested and should be considered as a reserve drug for treatment of infections with MDR Gram positive and Gram negative infections including anaerobes (Kelesidis et al., 2008).

Table.1 Microscopic colony morphology and biochemical characters of clinical isolates

Reactions/Organisms	<i>E. coli</i>	<i>K. pneumoniae</i>	<i>P. aeruginosa</i>	<i>A. baumannii</i>	<i>P. vulgaris</i>
Gram staining	-Ve rod	-Ve rod	-Ve rod	-Ve rod	-Ve rod
Motility	+	-	+	+	+
Indole	+	-	+	-	+
Methyl Red	+	-	-	-	+
Voges Proskauer	-	+	-	+	+
Citrate	+	+	-	+	+
H ₂ S Production	-	-	+		+
oxidase	-	+	+	-	+
Nitrate	-	-	+	-	-
Urease	-	+	-	+	+
Glucose	+	+	+	+	+
Sucrose	-	+	-	-	+
Lactose	+	+	-	-	-

Note where: -Ve rod stands for gram negative rods, + stands for positive reactions and – stands for negative reactions.

Table.2 The clinical isolates showed the antibiotic sensitivity reactions against various antibiotics

Antibiotics/Organisms	<i>E. coli</i> N=105	<i>K. pneumoniae</i> N=41	<i>P. aeruginosa</i> N=40	<i>A. baumannii</i> N=14	<i>P. vulgaris</i> N=5
Ceftazidime (Caz-30µg)	38% S	73% S	30% S	50% S	60% S
Gentamicin (G-10µg)	57% S	60% S	87% S	92% S	80% S
Tobramycin (Tob-10µg)	66% S	53% S	75% S	78% S	20% S
Amikacin (Ak-30µg)	47% S	70% S	50% S	21% S	80% S
Netilmicin (Net-30µg)	52% S	56% S	63% S	35% S	60% S
Tetracycline (Te-30µg)	90% S	80% S	87% S	57% S	80% S
Imipenem (Ipm-10µg)	97% S	85% S	67% S	64% S	60% S
Meropenem (Mem-10µg)	43% S	58% S	63% S	60% S	40% S
Polymixin (Poly-10µg)	95% S	87% S	90% S	85% S	80% S
Tigecycline (Tige-10µg)	100% S	100% S	97% S	100% S	100% S

Note where: S– Antibiotic sensitivity percentages of an antibiotic against 105-*Escherichia coli*, 41-*Klebsiella pneumoniae*, 40-*Pseudomonas aeruginosa*, 14-*Acinetobacter baumannii* and 5-*Proteus vulgaris* gram negative bacteria.

The present study shows tigecycline and polymyxin B are the potent antimicrobial agents against ESBL producing *Enterobacteriaceae* and multi-drug resistant *Acinetobacter baumannii*. Due to its long half-life and large volume of distribution, it can be an important lifesaving agent in the treatment of polymicrobial intra-abdominal, skin and soft tissue infections. It is not useful in bloodstream infections and nosocomial pneumonia. In view of its excellent activity against MDR pathogens, it is prudent to reserve Tigecycline and polymyxin B for life threatening infections when other options fail.

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