Original Research Article

Prevalence and Susceptibility Analysis of Gram negative pathogens in Tertiary Care Hospital

Abstract

Background and objective

Large amounts of antibiotics consumed by the human population has resulted in the culmination of pathogenic bacteria resistant to multiple drugs. The resistance profile of pathogens differ from one geographical location to another and keeps on changing continuously.

Methods

A retrospective observational analysis of antibiogram data was performed to characterize the susceptibility pattern of different pathogen isolates from various clinical sources. A total of 213 clinical isolates identified from the period June 2015 to June 2016 were included in the study.

Results

Of the 213 Gram negative isolates, 36.6% were from urine, 23.9% from respiratory specimens, 11.74% from blood, 10.33% from pus whereas 17.37% were from other sources. *E. coli* (42.25%) was most predominant pathogen isolated followed by *K. pnuemoniae* . (25.35%) and *Pseudomonas spp.* (15.96%) while other gram negative pathogens contributed 16.4%. Antibiogram analysis has shown CSE-1034 as the most susceptible drug exhibiting 91.1%, 77.8%, 82.4% and 82.3% susceptibility against *E. coli, K. pneumoniae*, *A. baumannii* and *P. aeruginosa.* Among carbapenems, both meropenem and imipenem-Cilastin were most susceptible against *E. coli*. Meropenem was least susceptible against *K. pneumoniae* (50%) and imipenem against *P. aeruginosa* (32.35%).

Like imipenem, Pip-taz was highest susceptible against *E. coli* (20%) and lowest against *P. aeruginosa* (26.47%).

Conclusion

Susceptibility profile indicates CSE-1034 (a novel antibiotic resistance breaker) as the most susceptible drug among all the classes of antibiotics against the gram-negative pathogens. A high resistance to piperacillin-tazobactam and penems, advocates use of CSE-1034 as empiric drug of choice in treatment of bacterial infectious diseases where the pathogen isolates are suspected resistant towards β-lactam and β-lactamase inhibitor combinations.

Keywords: Antibiotic, Clinical isolates, CSE-1034, Prevalence, Susceptibility, Resistance.

1. Introduction

The emergence of resistance among pathogenic bacteria towards potent antimicrobial agents has become a critical problem in modern medicine [1]. WHO has warned that the level of resistance to drugs used to treat common infectious diseases is arriving at a crisis point and if not controlled, entire population could be wiped out by these superbugs [2]. The developing resistance towards currently available drugs increases the economic burden on the community by increasing the rates of hospitalization, length of hospital stays and cost of treatment [3] [4][5]. The rising antimicrobial resistance among the most common opportunistic gram negative pathogens, are also associated with increased mortality and morbidity rates [2].

β-lactam antibiotics used to be the most common treatment for bacterial infections but the constant exposure of bacteria to β-lactams drugs has created a selective pressure leading to ESBL and MBL producing strains. [6]. In past few years, a significant increase

in the prevalence of ESBL and MBL producing strains has been observed throughout the globe [7]. These ESBL and MBL producing Gram-negative pathogens are reported resistant to other classes of antibiotics also [12,13].

Taking into account such a situation, there is a need to optimize the antibiotic therapy against multidrug-resistant pathogens which may vary from one geographical locale to another. Surveillance data and hospital antibiogram profiles help clinicians in the prescription of appropriate antimicrobial therapy. Therefore, we aimed to study the susceptibility profile of clinical isolates collected from Noble Hospital, Pune towards commonly used 2nd line antibiotics Ceftriaxone/Sulbactam/EDTA, β-lactam and β-lactamase inhibitor combination (Piperacillin-tazobactam) and Carbapenems (meropenem and imipenem-cilastatin) drugs.

2. Materials and Methods

2.1 Sample collection

Various clinical specimens used for pathogen isolation included urine, stool, blood, pus, endotracheal tube secretions (ETT), tracheal tube (TT) secretions, sputum, wound, , gall bladder specimens, abscess, drain, ear swab, vitreous eye, abdominal fluid, vitreous fluid, semen, peritoneal fluid and tissue specimens collected from 347 infected patients at Noble Hospital, Pune (India), during the period of July 2016 to February 2017. The collection and processing of the samples were done as per common standard operating procedures of hospital.

2.2 Isolation and Identification of microbes

All the samples were collected aseptically in sterile containers and inoculated on the different selective and non-selective culture media as per the standard microbiological techniques. Details of the culture media used for the isolation of pathogens from various clinical samples are given in Table 1. Blood samples were collected in Bactec bottles and

incubated in Bactec machine. These samples were further sub-cultured on the selective or non-selective media and incubated aerobically overnight at 37°C. Organisms were identified on the basis of colony morphology, gram staining, motility and biochemical reactions. Biochemical reactions were performed by inoculating the bacterial colony in a nutrient broth at 37°C for 2– 3 hours [15].

2.3 Antibiotic susceptibility testing

Antimicrobial susceptibility study was performed by Kirby–Bauer disk diffusion method as recommended by the Clinical Laboratory Standards Institute (CLSI) guidelines [16]. In brief, inoculum of 0.5 McFarland standards turbidity was prepared in a Mueller-Hinton broth (MHB, Hi-Media, Mumbai, India) from isolated colony of pathogens selected from 18–24 hour agar plates. A sterile cotton swab was dipped into the inoculum and streaked many times on the dried surface of a Mueller-Hinton agar (MHA) plate. After 5 minutes, antibiotic discs were applied and pressed down to check absolute contact with agar surface. The discs were apportioned in a minimum distance of 24 mm from the center. The plates were then incubated for 16-18 hrs aerobically at 37° C. The discs of meropenem (10 µg), imipenem-cilastatin (20 µg) and piperacillin-tazobactam (110 µg) were obtained from Microexpress Goa, India and CSE-1034 (45 µg) was obtained from third party.

3. Results

A total of 47 clinical specimens were obtained from the suspected patients out of which 213 (61.38%) clinical samples tested positive for gram negative pathogens. Out of these 213 Gram negative isolates, the maximum isolates were obtained from urine specimens (36.62%) followed by respiratory specimens (13.62%), blood (11.74%), pus (10.33%) and wound (6.10%) while all other samples contributed a total of 6.58% (Table 2).

On the basis of morphological and biochemical screening, eight bacterial species were obtained including *E. coli*, *K. pneumoniae, P. aeruginosa*., *A. baumannii*, along with other less prevalent gram negative bacilli such as *Proteus spp.*, *Salmonella spp.*, *Serratia spp*. and *Enterobacter spp*. which contributed 3.76% (% cumulatively) to the total clinical isolates. The detailed profile of various pathogens isolated from clinical specimens is shown in Table 3.

Table 2 represents the prevalence of different clinical isolates in different samples. Data revealed the maximum prevalence of *E. coli* in urine samples, pus and stool samples. *K. pneumoniae* was mostly isolated from blood and respiratory specimens whereas *P. aeruginosa* isolates were mostly retrieved from wound and respiratory specimens. *A. baumannii* was least prevalent in all the specimens.

Susceptibility profile of pathogens isolated from clinical specimens is presented in Table 4. Overall, 85.4% (182) of the total number of isolates were reported susceptible to CSE-1034, 59.6% (127) to Pip-taz, 66.2% (141) to Meropenem and 64.8% (138) to Imipenem. The susceptibility rates of CSE-1034 were *E. coli* (91.9%)*, K. pneumoniae* (77.8%)*, A. baumannii* (82.4%) and *P. aeruginosa* (82.3%). Among all the antibiotics tested, the least susceptibility was reported to Pip/Taz. *E. coli* exhibited the highest susceptibility (80%) to Pip-taz whereas the lowest was reported by *P. aeruginosa* (26.47%). Among Carbapenems, almost similar susceptibility of meropenem and imipenem-cilastin was reported against *E. coli* (77-82%) and *K. pnuemoniae* (48-50%). The meropenem was marginally better than imipenem-cilastin against *A. baumannii* (58.8% vs 52.9%) whereas was reported significantly better than imipenem-cilastin against *P. aeruginosa* (58.8% vs 32.3%).

Discussion

The predominant species isolated was *E. coli* (42.2%) followed by *K. pneumoniae* (25.3%). A good number of studies have reported *E. coli* and *K. pneumoniae* as most common and opportunistic clinical pathogens [17, 18]. Similar results with high prevalence of *E coli* (54.9%) were reported by Sikka *et al.* [19]. Sachdeva [20] has also reported the prevalence of *E. coli* to a tune of 51.7%. A similar prevalence of *K. pneumoniae* has been reported by Makkar *et al.* [21] who demonstrated 22% of *K. pneumoniae* from clinical isolates. Sahu *et al.* [22] reported the prevalence of *K. pneumoniae* to a tune of 32% which sustains our data. *Pseudomonas spp*. (15.9%) also contributed significantly to the isolated pool of pathogens. As compared to other studies, less number of *A. baumannii* isolates were identified in this study. The similar prevalence pattern of *Proteus spp.*, *Salmonella spp.*, *Serratia spp.* and *Enterobacter spp* is also reported by many other studies. [17, 24, 25]

Similar to our observations, Ruppe et al. [14] have also reported 90% prevalence of *E. coli* in stool samples. Majority of *E. coli* (54%) isolates were recovered from urine during the study performed by Kumar et al. [17]. Ibrahim et al. [26] have also reported 40-50% prevalence of *E. coli* in pus samples. *K. pneumoniae* isolates were mostly isolated from blood and respiratory specimens whereas *P. aeruginosa* was mostly isolated from wound and respiratory specimens.

Among all the antibiotics tested, the least susceptibility was reported to Pip/Taz and highest was reported towards CSE-1034. A high rate of resistance observed to Pip/taz which is normally recommended second line of treatment in our hospital could be possibly the indiscriminate consumption of pip-taz. The AMR surveillance study conducted in India has shown resistance against pip-taz has risen to 65-70% [19]. Among carbapenems, the average susceptibility rates were 65% against all the pathogen isolates. The emergence of

carbapenem-resistant strains, which ranges from 18-68% in different isolates is a matter of big concern as carbapenems are considered as the last resort drugs for MDR bacterial infections. Singh *et al*. [11] have reported that MBLs to a tune of 15-22% among the Gram-negative isolates in their study.

The high rate of carbapenem resistant strains reported in this surveillance study is a matter of grave concern and needs to be addressed on priority at the global level. One of the approaches that the clinicians have adopted to reduce selective pressure on last resort drugs is by pumping in the use the antibiotic resistance breakers "ARBs" along with antibiotics to revive them for clinical purposes. CSE-1034 is one such combination of beta-lactam/beta-lactamase inhibitor (BL/BLI) combination with ARB "EDTA". Interestingly, a significant number of isolates were sensitive to CSE-1034 i.e., *E. coli* (98.8%), *K. pneumoniae* (90.5%), *P. aeruginosa* (89.9%) and *Acinetobacter spp* (81%). Surprisingly, 131 isolates reported as Meropenem resistant were susceptible to CSE-1034 (Table 4). The higher susceptibility of gram-negative pathogens to CSE-1034 has been reported by several other studies also. CSE-1034 is a novel combination of Ceftriaxone, Sulbactam and disodium edetate and the high susceptibility of CSE-1034 could be attributed to the synergistic effect of Ceftriaxone, disodium edetate and Sulbactam. The non-antibiotic adjuvant, EDTA mediates various antimicrobial effects by enhancing the penetration of antibiotic into cell membrane, decreases over-expression of efflux pumps, bio-film eradication, de-activates carbapenemases-MBL by chelating Zinc ions.

About last line therapy agents for MDR infections, our study has shown Carbapenems as the most active agent only against *E.coli* (82%). Around 36-45% of *P. aeruginosa* and 45% of *Acinetobacter* spp. were Carbapenem resistant. Resistance to meropenem was found highest in *Klebsiella spp.* (54%). Chauhan *et al.* [19] have reported a Carbapenem resistance of 14.6% in *E. coli* and 29.6% in *Klebsiella spp.* in hospital isolates from various in and

175 outpatient areas. Gupta *et al*. [21] have reported a Carbapenem resistance ranging from 17-
176 22% in different strains of Enterobacteriaceae from North India.

177 Based on pathogen type, *E. coli* exhibited the highest susceptibility rate whereas the
178 lowest was reported against *P. aeruginosa. E. coli* was found to be most susceptible clinical
179 isolate among major pathogens which displayed 80%, 75.5% and 82.2% sensitivity against
180 piperacillin-tazobactam, meropenem and imipenem-cilastatin respectively. Correspondent
181 results were observed by many authors who reported 100%, 90% and 96.5% sensitivity of *E.*
182 *coli* against meropenem, piperacillin-tazobactam and imipenem-cilastatin respectively
183 [35,36]. *Klebsiella spp*. exhibited intermediate susceptibility i.e. 44.4%, 50% and 48.1%
184 towards piperacillin-tazobactam, meropenem and imipenem-cilastatin. Similar results were
185 noted by many authors who revealed 40-60% sensitivity of *Klebsiella spp.* against
186 piperacillin-tazobactam, meropenem and imipenem-cilastatin [37,38]. As reported earlier
187 also, *Acinetobacter spp*. experienced highest susceptibility (96.3%) towards CSE-1034CSE-
188 1034 only while extreme resistance (96.3% each) against rest of the antibiotics which is due
189 to sulbactam (a β-lactamase inhibitor) which owns intrinsic whole-cell activity against
190 *Acinetobacter spp.* [39]. Surprisingly, contrary to expectations, *Pseudomonas spp.*
191 documented 73.5%, 73.5% and 67.6% resistance against piperacillin-tazobactam, meropenem
192 and imipenem-cilastatin respectively. Mohammadi and Feizabadi [37] reported >60%
193 resistance of piperacillin+tazobactam against gram negative bacilli isolated from clinical
194 samples which supports our data. Similarly, Hout *et al.* [40] revealed 70-100% resistance of
195 meropenem towards *Acinetobacter spp.* and *Pseudomonas spp.*. Likewise, Shour and El-
196 Sharif, [36] and Eldomany and Abdelaziz noticed significant resistance (>50%) of
197 imipenem-cilastatin in *Acinetobacter spp.* and *Pseudomonas spp.* which is in accordance with
198 our present data [41].

The emergence of antimicrobial resistance against BL-BLI and carbapenem drugs is due to numerous elements which assists the scattering of resistance among clinical pathogens which includes production of MBL enzymes, biofilm formation, over expression of efflux pumps and accumulation of the drug [42,43]. None of these mechanisms are dressed by either piperacillin-tazobactam, meropenem or imipenem-cilastatin and probably this could be one reason for CSE-1034 super performance that it s supplemented with EDTA as ARB. The progressive and relentless resistance towards BL-BLI and carbapenem antibiotics is probably the result of overuse of antibiotics, improper processing and inappropriate prescribing [44]. In the light of above discussion it is evident that Antibiotic adjuvant therapy which has ARB can be used as the prime choice of therapeutics to overcome the resistance raised among gram negative pathogens towards β-lactam and β-lactamase inhibitor combinations and penems in the treatment of bacterial infectious diseases.

4. Conclusion

This retrospective study indicates the rise in resistance among most prevalent and opportunistic gram negative pathogens against β-lactam and β-lactamase inhibitor combinations and penems. Present data strongly advocates precedence of CSE-1034 over β-lactam and β-lactamase inhibitor combinations and penems as CSE-1034 has scored 85-100% susceptibility which excels the antimicrobial activity of rest of the drugs. Therefore, CSE-1034, a novel product with antibiotic resistance breaker can be used as an empirical and alternate choice of drug over potent therapeutics in encountering multidrug resistance among healthcare-associated pathogens.

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Table 1: Selective culture medium used for isolation of different pathogens.

Pathogen	Selective media
Klebsiella spp.	Hicrome Klebsiella selective agar base medium
E. coli	Eosine Methylene Blue (EMB) agar medium
Acienetobacter spp.	Leeds acinetobacter agar base medium
Pseudomonas spp.	Citrimide agar medium
Proteus spp.	EMB agar medium
Salmonella spp.	Wilson and Blair bismuth sulphite medium
Serratia spp.	Caprylate-thallous agar medium
Enterobacter spp.	EMB agar medium

Table 2: A profile of clinical samples used as a source of the pathogenic isolates.

Sr. No.	Name of clinical samples	Total no. of samples collected	Number of samples showing growth of pathogens (%)	Number of samples not showing growth of pathogens
1	Urine	94	78 (36.62%)	16
2	Respiratory specimens	84	51	
3	Blood	39	25 (11.74%)	14
4	Pus	34	22 (10.33%)	12

5	Wound	22	13 (6.10%)	9
6	Stool	19	10 (4.69%)	9
7	Other samples	55	14 (6.58%)	41
	Total	**347**	**213 (61.38%)**	**134**

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353 **Table 3: Prevalence of different clinical isolates in different samples.**

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	Clinical Isolates					
Samples	**No. of isolates**	***E. coli* (%)**	***Klebsiella spp.* (%)**	***Acinetobacter spp.* (%)**	***Pseudomonas spp.* (%)**	**Other pathogens (%)**
Urine	78	59 (75.64)	11 (14.10)	0	7 (8.97)	1 (1.28)
Respiratory specimens	51	6 (3.45)	17 (31.03)	10 (44.83)	12 (23.52)	6 (11.76)
Blood	25	2 (8)	10 (40)	4 (36)	5 (20)	4 (16)
Pus	22	10 (45.45)	6 (27.27)	2 (9.09)	2 (9.1)	2 (9.1)
Wound	13	2 (15.38)	3 (23.08)	1 (7.69)	5 (38.46)	2 (15.38)
Stool	10	8 (80)	2 (20)	0	0	0
Other samples	14	3 (21.43)	5 (35.71)	0	3 (21.43)	3 (21.43)
Total	**213**	**90**	**54**	**17**	**34**	**18**
Total (%)		**42.25 %**	**25.35 %**	**7.98 %**	**15.96 %**	**8.45%**

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356 **Table 4: Susceptibility pattern of clinical isolates.**

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Susceptibility (%)					
Clinical isolates		**AAE**	**BL-BLI**	**Carbapenem**	
	No. of isolates	**CSE-1034**	**Piperacillin-tazobactam**	**Meropenem**	**Imipenem-cilastatin**

		S	**R**	**S**	**R**	**S**	**R**	**S**	**R**
E. coli	**90**	91.1 (82)	8.9 (8)	(80) 72	(20) 18	77.7 70	22.3 22	82.22 74	17.78 16
Klebsiella spp.	**54**	77.8 (42)	22.2 (12)	(44.44) 24	55.56 30	50 27	50 27	48.15 26	51.85 28
Acinetobacter spp.	**17**	82.4 (14)	17.6 (3)	35.3 6	64.7 11	58.8 10	41.2 7	52.9 9	47.1 8
Pseudomonas spp.	**34**	82.3 (28)	17.7 (6)	26.47 9	73.53 25	58.8 20	41.2 14	32.35 11	67.65 23
Other pathogens	**18**	(88.9) 16	(9.91) 2	88.9 16	9.91 2	88.9 16	9.91 2	100 18	0

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