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ORIGINAL ARTICLE

Prevalence of multidrug resistant and extended spectrum beta-lactamase producing *Pseudomonas aeruginosa* in a tertiary care hospital

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Abstract Resistance to broad-spectrum beta-lactams, mediated by extended-spectrum beta-lactamase enzymes (ESBL), is an increasing problem worldwide. The present study was undertaken to determine the incidence of ESBL-production among the clinical isolates of *Pseudomonas aeruginosa* and their susceptibility to selected antimicrobials. A total of one eighty-seven clinical specimens were tested for the presence of ESBL production using the double-disc synergy test. Of these, 25.13% ($n = 47$) isolates of *P. aeruginosa* were observed as ESBL positive. The maximum number of ESBL-producing strains were found in sputum (41.67%; $n = 24$) followed by pus (28.36%; $n = 19$), cerebrospinal fluid and other body fluids (21.74%; $n = 5$), urine (20.45%; $n = 9$) and blood (13.79%; $n = 4$). ESBL producing isolates exhibited co-resistance to an array of antibiotics tested. Imipenem and meropenem can be suggested as the drugs of choice in our study.

1. Introduction

The worldwide emergence of multi-drug resistant bacterial strains in hospitals and community continues to be a problem of due scientific concern, especially infections caused by *Pseudomonas* species and *Pseudomonas aeruginosa* in particular. *P. aeruginosa* is an opportunistic pathogen with inherent resistance to many antibiotics and disinfectants including anti-pseudomonal Penicillins, Ceftazidime, Carbapenems, Aminoglycosides and Ciprofloxacin (Dundar and Otkun, 2010).

P. aeruginosa is physiologically versatile and flourishes as a saprophyte in multiple environments, including sinks, drains, respirators, humidifiers and disinfectant solutions. Infections due to *P. aeruginosa* are seldom encountered in healthy adults; but in the last two decades, the organism has become increasingly recognized as an aetiological agent in a variety of serious

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Table 1 ESBL-producing *P. aeruginosa* strains isolated from various samples.

S. No	Specimen type	No. of *P. aeruginosa* strains(n = 187)	ESBL positive *P. aeruginosa* strains (n; %) (n = 47)
1.	Urine	44	09 (20.45%)
2.	Blood	29	04 (13.79%)
3.	Pus	67	19 (28.36%)
4.	Sputum	24	10 (41.67%)
5.	Cerebrospinal fluid body fluid and other	23	05 (21.74%)

infections in hospitalized patients, especially those with impaired immune defenses (Shahid et al., 2003).

Extended-spectrum beta-lactamases (ESBLs) have emerged as an important cause of resistance in Gram-negative bacteria. Beta-lactam antibiotics are among the safest and most frequently prescribed antimicrobial agents all over the world in treating Gram positive and Gram negative infections (Bradford, 2001). Production of beta-lactamases is the most common mechanism of bacterial resistance to these antibiotics. These enzymes are numerous and are plasmid mediated, capable of hydrolysing and inactivating a wide variety of beta-lactam antibiotics. In addition, ESBL producing organisms exhibit co-resistance to many other classes of antibiotics resulting in the limitation of therapeutic options. For this reason, ESBL–mediated infections have been increasingly reported worldwide (Khanfar et al., 2009).

The aim of the present study was to determine the incidence of ESBL-production among the clinical isolates of *P. aeruginosa* and their susceptibility to antimicrobials.

2. Methods

2.1. Bacterial isolates

One hundred eighty-seven isolates of *P. aeruginosa* were recovered from various clinical specimens. These included 44 isolates from urine, 29 isolates from blood, 67 isolates from pus, 24 isolates from sputum and 23 isolates from cerebrospinal fluid and other body fluids. All the specimens were quickly sent to the laboratory to be processed. Standard methods for isolation and identification of these bacteria were used (Cowan and Steel, 1970).

2.2. Antimicrobial susceptibility testing and ESBL detection

Antimicrobial susceptibility testing of the isolates was performed by the standard disc diffusion method as recommended by the Clinical and Laboratory Standards Institute (CLSI, 2008). *P. aeruginosa* ATCC 27853 was used as a control strain. ESBL production was detected by double disc synergy test as described by Jarlier et al. (1998). Synergy was determined between a disc of amoxyclav (20 μg amoxycillin and 10 μg clavulanic acid) and a 30 μg disc of each third generation cephalosporin test antibiotic placed 15 mm apart on a lawn culture of the isolate under test on Mueller Hinton agar plates. The test organism was considered to produce ESBL if the zone size around the antibiotic disc increased towards the amoxyclav disc. This increase occurs as the clavulanic acid present in the amoxyclav disc inactivates the ESBL produced by the test organism.

3. Results

A total of 187 bacterial isolates were analysed from various clinical specimens. Of these, 25.13% (n = 47) *P. aeruginosa* strains were found to be ESBL positive. The highest number of ESBL-producers were isolated from sputum (41.67%) followed by pus (28.36%), cerebrospinal fluid and other body fluids (21.74%), urine (20.45%) and blood (13.79%) (Table 1). Antibiotic susceptibility tests were performed for ESBL producing isolates. Imipenem and meropenem were found to be the most effective antibiotics against the ESBL-producing *P. aeruginosa* isolates. Zero percent resistance was found against both imipenem and meropenem. Among the third-generation cephalosporins, the highest resistance was found against ceftazidime, which was 91.49% (n = 43). The percent resistance to selected antimicrobials exclusively among the ESBL-producing isolates has been shown in Table 2.

4. Discussion

P. aeruginosa is a leading cause of nosocomial infections, including pneumonia, urinary tract infections, and bacteremia. The infections can be particularly severe in patients with impaired immune systems, such as neutropenic or cancer patients (Pagani et al., 2004). Infections caused by *P. aeruginosa* are difficult to treat as the majority of isolates show varying degrees of inherent resistance. Acquired resistance is also reported by the production of plasmid mediated AmpC beta (beta)-lactamase, ESBL and metallo beta-lactamase enzymes (Manchanda and Singh, 2008).

Table 2 Percent resistance to the selected antimicrobial agents among the ESBL-positive *P. aeruginosa* isolates (n = 47).

S. No.	Antibiotics	*S*	*R*	%*R*
1	Ampicillin	00	47	100.00
2	Aztreonam	11	28	59.57
3	Amikacin	07	40	85.11
4	Cefotaxime	10	37	78.72
5	Ceftazidime	04	43	91.49
6	Cefazolin	13	34	72.34
7	Cefepime	16	31	65.96
8	Cefoperazone	15	32	68.09
9	Ceftriaxone	08	39	82.98
10	Ciprofloxacin	12	35	74.47
11	Piperacillin	18	29	61.70
12	Tigecycline	14	33	70.21
13	Gentamicin	02	45	95.74
14	Imipenem	47	00	00.00
15	Meropenem	47	00	00.00

S = Sensitive, R = Resistant.

In the present study we observed that 25.13% ($n = 47$) *P. aeruginosa* were ESBL producers. The frequency of ESBL producing isolates was highest in sputum (41.67%) followed by pus (28.36%), cerebrospinal fluid and other body fluids (21.74%), urine (20.45%) and blood (13.79%). This is in harmony with the findings of Aggarwal et al. (2008).

The ESBL producing *P. aeruginosa* isolates exhibited co-resistance against most of the antibiotics tested. This is consistent with most of the recent findings (Bandekar et al., 2011; Begum et al., 2013). All ESBL producing *P. aeruginosa* isolates were sensitive to imipenem and meropenem. This is in harmony with the findings of Okesola and Oni (2012).

The introduction of carbapenems into clinical practice represented a great advance for the treatment of serious bacterial infections caused by beta-lactam resistant bacteria. Due to their broad spectrum of activity and stability to hydrolysis by most beta lactamases, carbapenems have been the drug of choice for treatment of infections caused by penicillin or cephalosporin-resistant Gram-negative bacilli especially, ESBL producing Gram-negative infections (Mendiratta et al., 2005).

5. Conclusion

The present study determines the prevalence of ESBL producing *P. aeruginosa* with limited susceptibility to antimicrobials in hospital environment. In order to combat these problems proper antibiotic policies should be formulated. Further, it was observed that all the ESBL-producing isolates were susceptible to imipenem and meropenem. This brings due relief as these are the drugs of choice in the treatment of *Pseudomonas* infection.

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