ESBL producing Pseudomonas aeruginosa in clinical specimens: Is it a scary nightmare or paper tiger?

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Abstract

Background: Pseudomonas aeruginosa well known as opportunistic pathogen, has been implicated in life threatening nosocomial infections in recent years. This can be attributable to acquired resistance by plasmid mediated different types of extended spectrum beta lactamases(ESBL). The present study was undertaken to detect the ESBL production of Pseudomonas aeruginosastrains and analyze their susceptibility pattern.

Methods: Various clinical specimens received in our laboratory were processed and Pseudomonas aeruginosa was identified as per standard microbiological procedure. A total of 132 clinical isolates of Pseudomonas aeruginosa identified during the study period were included in the study. All isolates were subjected for ESBL screening test. Potential ESBL producer was then subjected for ESBL Phenotypic confirmatory test –Disc Diffusion method(PCDDT). Antimicrobial susceptibility test was performed by Kirby – Bauer disc diffusion method on all confirmed isolates by PCDDT method as per Clinical Laboratory Standard Institute (CLSI 2016) guidelines.

Results: By phenotypic confirmatory method, the frequency of occurrence of ESBL among the isolates identified was 21.96% (n = 29).The highest number of ESBL producers was obtained from urine samples(27.7%) followed by respiratory infection (23.68%) and wound infection (22.95%). In-vitro susceptibility of ESBL producers revealed high level of resistance to third generation cephalosporin. Low resistance was observed to Imipenem (3.4%) and Piperacillin – Tazobactam (13.79%).

Conclusion: Our study highlights the unique challenge imposed by Pseudomonas aeruginosa as the therapeutic choices are limited by ESBL production. To overcome this issue, we recommend routine ESBL detection and surveillance of antibiotic resistance in hospital settings.

Keywords: Pseudomonas aeruginosa, ESBL, Third generation cephalosporins, Imipenem, Urine, ESBL Screening test.

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Introduction

Emergence of resistant bacteria has been posing severe threat and therapeutic challenge to the physicians especially in intensive care units. In the recent past, gram negative bacilli are found to be tough adversaries for clinicians, by displaying various mechanism of resistance which includes ESBLs, Amp C and MBL.

In particular, Pseudomonas aeruginosa well known opportunistic pathogen, has been implicated in life threatening nosocomial infections due to its inherent resistance to many antibiotics and development of increased(particularly multidrug) resistance in healthcare settings. This can also be attributable to acquired resistance by plasmid mediated Amp C beta lactamase, extended spectrum beta lactamase and metallobeta lactamase enzymes[(1)].

The Extended spectrum betalactamases (ESBLs) which hydrolyze oxyimino beta lactams which include cefotaxime, ceftriaxone, ceftazidime and aztreonam but yet inhibited by beta lactamase inhibitors such as clavulanic acid have been reported increasingly among the members of Enterobacteriaceae.

Recently, ESBL producing Pseudomonas aeruginosa has been detected and increasingly reported in most parts of the world. ESBL enzymes reported in Pseudomonas aeruginosa are of different types.The ß-lactams PER-1 (Pseudomonas extended spectrum betalactamase) was the first ESBL (classA,) identified and fully characterized in P.aeruginosa in1993. Another unrelated ESBL from P.aeruginosa, i.e. the ß-lactamase VEB-1, was originally identified in Escherichia coli and Klebsiella isolates, it was also subsequently found in P.aeruginosa strains from Thailand, Kuwait and China[(2)]. VEB and PER types were found to be the most common (or least rare) ESBL in P. aeruginosa in several countries, contrasting to the dominance of CTX-M, SHV, and TEM ESBL in Enterobacteriaceae.

The TEM-type enzymes described in P.aeruginosa are TEM-4, TEM-21, TEM-24, and TEM-42, in rare isolates from France. Few outbreaks of SHV producing Pseudomonas aeruginosa have been reported (SHV-2a) in France and (SHV-12) in Thailand. Several

oxacillinases (OXA-2 and OXA-10 derivatives and OXA-18) that have extended substrate profiles, including extended spectrum cephalosporin, have been reported in P. aeruginosa.OXA-ESBLs are mutants of OXA-2 and OXA -10, belonging to the molecular class D of Ambler's scheme and 2d of functional group under Bush-Jacoby-Medeiros classification whereas the other ESBL belongs to class A. Recently, P. aeruginosa producing OXA-4 ESBL for the first time in the Indian subcontinent was also reported[3].

Being plasmid mediated, they are easily transmitted to other members that would favor not only the dissemination of resistance to betalactams but also to other commonly prescribed antibiotics such as Aminoglycosides, Sulphonamides. This is due to the fact that plasmid of such strain often carries resistance genes to various antibiotics along with ESBL gene[4].

As routine antimicrobial susceptibility testing in our laboratory fails to detect the production of ESBL enzymes, ESBL producing strains are probably more prevalent than currently recognized. As a consequence, therapeutic failure as they might show false susceptibility in routine laboratory testing in spite of enzyme production, prolonged illness and prolonged hospitalization might ensue.

Delayed therapy in pseudomonas aeruginosa infections have been well correlated with increased mortality[5]. High mortality rates in serious infections could be explained by the fact that the antibiotics were chosen empirically until the organism is isolated and tested for ESBL production. Prompt and accurate detection of ESBL producing P.aeruginosa is crucial for optimal treatment of critically ill and hospitalized patients and also, to control the spread of resistance.

Hence knowledge about the antibiogram pattern of current strains would be helpful for the clinicians to choose appropriate antibiotics

Hence, the present study was conducted with an objective to find the prevalence of ESBL producing P. aeruginosa. We also aimed to detect the resistant profile of ESBL producing P. aeruginosa.

Materials and Methods

Study design: Prospective study

Study period: 1 year

Various clinical specimens which included urine, blood, sputum, pus, CSF and other body fluids received in our laboratory were processed and Pseudomonas aeruginosa was identified as per standard microbiological procedure[6]. A total of 132 clinical isolates of Pseudomonas aeruginosa identified during the study period were included in the study.

As current interpretations of clinical laboratory standard institute for disc diffusion do not identify ESBL strains as 'resistant', all isolates were screened and subsequently confirmed for ESBL production.

Till date, no recommendations have been made by CLSI for detection of ESBL in Pseudomonas aeruginosa, so we employed the same criteria laid down for Enterobacteriaceae (CLSI, 2015), as the principle remains the same. CLSI 2015 has recommended the use of any of the following antibiotics for screening for ESBL producers. It includes antibiotic discs of ceftazidime, aztreonam, cefotaxime and ceftriaxone. We have used antibiotic disc of ceftazidime (30µg). All isolates were subjected for **ESBL screening test**[7].

Procedure

Inoculums with 0.5 McFarland standard turbidity was prepared from culture plates of Pseudomonas aeruginosa. MHA plates were then inoculated by lawn culture using a sterile cotton swab. With sterile forceps Ceftazidime was placed firmly and the plate was incubated at 37^0C for 18 -24 hrs. Isolates were considered a potential ESBL producer if the zone of inhibition for ceftazidime was observed to be <22mm.

Potential ESBL producer was then subjected for ESBL Phenotypic confirmatory test –Disc Diffusion method as recommended by Clinical Laboratory Standards Institute, 2015 [7].

Phenotypic confirm atorydisc diffusion test (PCDDT) for ESBL:

A Muller Hinton agar plate was taken and a lawn culture of potential ESBL producing Pseudomonas aeruginosa was made. Then ceftazidime (30µg) disc alone and with clavulanic acid (10µg) were placed at an appropriate distance from each other on the plate and incubated aerobically at 37^0C overnight. A $\geq$ 5mm increase in zone diameter for antimicrobial Ceftazidime tested in combination with clavulanic acid in comparison to the zone diameter when tested alone confirmed the organisms to be an ESBL producer by PCDDT.

Antimicrobial susceptibility test:

Antimicrobial susceptibility test was performed on all confirmed isolates by PCDDT method. Kirby – Bauer disc diffusion method as per Clinical Laboratory Standard Institute (CLSI 2015) guidelines was performed[7].

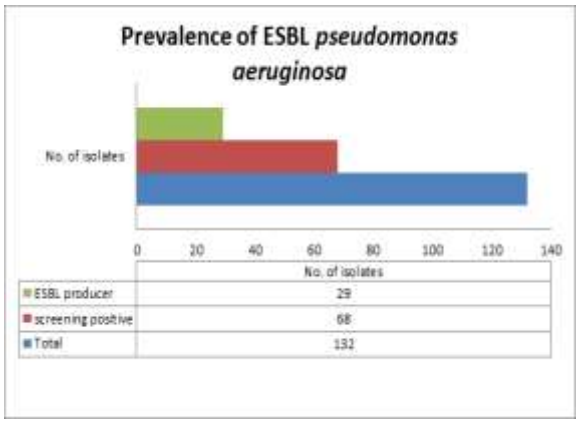
All the isolates were tested against the following panel of Antipseudomonal antibiotics of standard strengths. Commercially available antibiotic discs (Hi media Labs) were used for Antimicrobial susceptibility testing. Following antibiotic discs were used Amikacin (30µg), Gentamicin (10µg), Ciprofloxacin (5µg), Levofloxacin (5µg), Ofloxacin (5µg) Ceftazidime(30µg), Piperacillin (100 µg), Aztreonam (30µg), Piperacillin and Tazobactam (100/10 µg), Cefepime (30µg), Tobramycin(10µg). Results were interpreted according to the CLSI guidelines.

Results

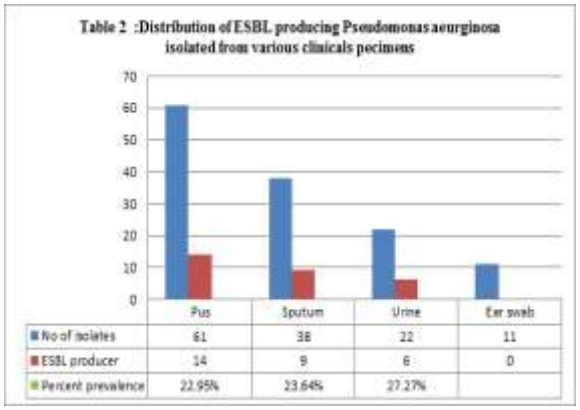
- A total of 132 non duplicate isolates of Pseudomonas aeruginosa identified during the

study period were analyzed. Pseudomonas aeruginosa was obtained from various clinical specimens such as Sputum, Pus, wound swab, Ear swab, etc.,

- Out of 132 isolates, only 68 isolates of Pseudomonas aeruginosa showed zone of inhibition ≤ 22 mm for third generation cephalosporin (3GC), Ceftazidime. Of these 42.64% (n = 29) of Pseudomonas aeruginosa isolateswere found to be ESBL producers. (Table 1). Prevalence of ESBL producing Pseudomonas aeruginosa (29/132) in this study was 21.96%.



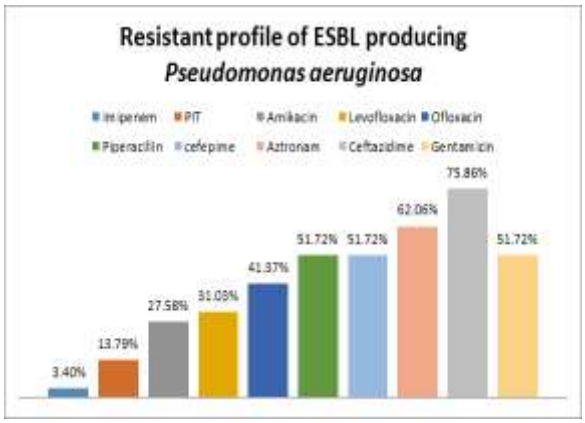
Pseudomonas aeruginosa has been increasingly associated with wound infections. Even in our study the maximum isolates (61) were obtained from pus/wound swab followed by sputum (38), Urine (22) and Ear swab(11). During the study period, all blood samples received was found to be culture negative for Pseudomonas aeruginosa.



The highest number of ESBL producers was obtained from urine samples(27.7%) indicating urinary tract infection (UTI) was the most common infection associated with ESBL producing Pseudomonas aeruginosa followed by respiratory infection (23.68%) and wound infection (22.95%). Surprisingly, isolates from Ear infections were not found to be ESBL producer.

Antimicrobial susceptibility tests were performed for all the ESBL producers Among the antipseudomonal antibiotics tested against the ESBL isolates, least resistance was observed for Imipenem (3.4%) followed by Piperacillin and Tazobactam (13.79%) suggesting that they can be considered as appropriate choice of drug when potential ESBL producer are suspected. In contrast, Piperacillin alone reported to have resistant percentage of 51.72%.

Among the fluoroquinolones tested, ofloxacin exhibited 41.37% of resistance compared to 31.03% of Levofloxacin. 75.86% of isolates was observed to be resistant to third generation Cephalosporins, Ceftazidime.



Discussion

In recent times, emergence of antibiotic resistance has threatened the effectiveness of many antibiotic agents and it is recognized as a public health threat. Pseudomonas aeruginosa which has particular propensity for drug resistance has been reported to be associated with increased mortality and morbidity[8].

By phenotypic confirmatory method, the frequency of occurrence of ESBL among the isolates identified was 21.96% (n=29). Aggarwal et al.,[9] and Shaikh et al.,[10] on similar study observed prevalence rate of 20.3% and 25.13% which is comparable to our findings. In contrast, high prevalence of ESBL producing Pseudomonas aeruginosa was also reported by Vijay Mane et al., Varun Goel et al., and Silpi Bask et al who observed 57%, 42.3% and 40% respectively[11,12,13]. This may be due to the fact that prevalence in any geographical area is influenced by the antibiotic policy of that place, the carriage rate among the hospital personnel and type of disinfectant used. Hence, prevalence of ESBL producing Pseudomonas aeruginosa varies from country to country and from centre to centre.

ESBL producing Pseudomonas aeruginosa were isolated from pus, sputum and urine. The highest percentage of ESBL (27.7%) was observed from urine specimens(urinary tract infection). This observation is of great concern because of severity of the UTI and if

not treated with appropriate antibiotics might lead to chronic illness. High rate of ESBL producers in common infections like UTI also implies that are no longer easy to treat with oral agents.

In contrast to our findings, a recent study by Pramodhini et al., reported high percentage from pus[14]. This might be due to the fact that type of infections associated with ESBL strains also varies from place to place, emphasizing the need for continuous surveillance irrespective of nature of specimens.

Among the 29 ESBL producer, 22 isolates were observed to be resistant to Ceftazidime (zone size ≤ 18 mm) according to CLSI 2016 guidelines. 7 (24.13%) of total ESBL confirmed isolates had shown false susceptibility to third GC Cephalosporin, Ceftazidime in spite of their ESBL production. This clearly specify the importance of detecting ESBL production at the earliest and reporting such isolates as resistance to all Penicillin, Cephalosporin and Aztreonam irrespective of their sensitive zone.

ESBL producers are found to colonize the gastrointestinal tract of infected patients for a long period of time which facilitate their spread through communities by fecal contamination of soil and water[15]. Sucha scenario depicts grave danger to the community as they are multidrug resistant in nature. To ward off the spread, judicious use of antibiotics and appropriate empirical choice of drug need to be given.

The results of antimicrobial susceptibility pattern of Pseudomonas aeruginosa isolates from our locality to ten antipseudomonal antibiotics showed that 22 strains were highly resistant to Ceftazidime (75.96%). Report of recent studies by Kaur et al.,(2013) & Zafer MM et al.,(2014) agreed with our study in observing high level resistance to ceftazidime[16,17]. However, in a study by Joseph NM et al.,[18] and resistance rate reported was 33%. This clearly suggests that Cephalosporins use should be limited in order to combat therapeutic challenge imposed by ESBL producers.

Most ESBL isolates also showed high resistance to otherbetalactam antibiotics tested which includes fourth generation Cephalosporins (Cefepime) (51.78%), Piperacillin (51.72%), Monobactams (Aztreonam)(62.06%). We have noted that ESBL producers were highly resistant even to Aminoglycosides (ranging from 27% to 51%) and Fluoroquinolones (ranging from 31% to 41%). Although ESBL do not have intrinsic effect on these group of antibiotics it is often due to co transfer of resistance to these antibiotic categories with plasmid mediating ESBLs. This phenomenon has been reported by other studies as well[19].

ESBLs are usually inhibited by β-lactamase inhibitors, such as clavulanic acid, sulbactam or tazobactam. Therefore, use of β-lactam/β-lactamase inhibitor combinations has been considered for the treatment of infections due to ESBL-producing organisms. Currently, carbapenems are generally regarded as the preferred agent for treatment of infections due to ESBL-producing organisms as they are resistant to ESBL-mediated hydrolysis. Our data revealed excellent In-vitro susceptibility of ESBL producers to Imipenem (3.4%) and Piperacillin – Tazobactam(13.79%).

Prevalence of ESBL isolates in our locality may be high as they are not routinely detected and reported especially for Pseudomonas aeruginosa. Detection of ESBLs warrants the use of appropriate choice of drugs to target, at the earliest.

Conclusion

Our study highlights the unique challenge imposed by Pseudomonas aeruginosa as the therapeutic choices are limited by ESBL production. To overcome this issue, we recommend routine ESBL detection and surveillance of antibiotic resistance in hospital settings.

Conflict of interest: None

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