Classification of drug resistance and novel single plate sensitivity testing to screen ESBL, AmpC, MBL in MDR, XDR and PDR isolates

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Abstract:
Objective: *Detection of drug resistant organisms and their co-existence at the right time and within a short period is one of the prime roles of a clinical microbiologist. This study was undertaken to classify drug resistance and to detect different mechanisms of resistance to beta lactam antibiotics in gram positive and gram negative bacteria by single plate sensitivity testing.*
Method: *The bacterial isolates were collected and all non-repeat resistant isolates were classified as Multi drug resistant (MDR), extensively drug resistant (XDR) and Pan drug resistant (PDR). Novel single plate sensitivity testing was done to detect extended spectrum beta-lactamases (ESBL), AmpC beta-lactamases (AmpC) and metallo-beta-lactamases (MBL) among MDR, XDR and PDR isolates.*
Result: *A total of 3176 clinical samples were obtained of which 472 and 756 were gram positive cocci (GPC) and gram negative bacilli (GNB) respectively. Among GPC 45% isolates were Methicillin resistant Staphylococcus aureus. The percentage of MDR was high in both GPC and GNB isolates when compared to XDR and PDR. Among the MDR isolates of GNB, 64% were ESBL producers, 8% were AmpC producers and coexistence of both ESBL and AmpC was found in 5%. Amongst the XDR isolates, 49% were ESBL and 21% were AmpC producers, 2% were MBL producers and13% were both ESBL and AmpC positive. In PDR isolates, 0.2% were MBL producers.*
Conclusion: *Previous literature documented increase of MDR isolates and recent studies have shown the emergence of XDR. It has to be controlled before it shifts to PDR.*
Keywords: *Drug resistance, MDR, XDR, PDR.*

I.   Introduction:

Drug resistance is rapidly prevalent among microorganisms and emergence of resistance to multiple antimicrobial agents in pathogenic bacteria has become a significant public health threat as there are fewer, or even sometimes no effective antimicrobial agents available for infections caused by these bacteria [1]. The resistance to antimicrobial agents by organisms are due to various mechanisms like enzymatic action, alteration of target site, alteration of metabolic pathways, efflux pump, acquisition of additional DNA elements, and degeneration, in which beta-lactamase activity is most common drug resistance mechanism among microorganisms. Bet- lactam antibiotics are commonly used as broad spectrum for both gram positive and gram negative bacteria [2]. In gram positive bacteria, Methicillin resistant Staphylococcus aureus shows beta lactamase activity, whereas in gram negative bacteria, extended spectrum beta-lactamases (ESBL), AmpC beta-lactamases, and metallo-beta-lactamases (MBL) are the common drug resistant mechanisms [3].

Different patterns of resistance are found in healthcare-associated infections. The resistance of a microorganism to antimicrobial agents can be classified into three categories: Multi drug resistant (MDR), extensively drug resistant (XDR) and Pan drug resistant (PDR). MDR is defined as acquired non-susceptibility to at least one agent in three or more antimicrobial categories, XDR is defined as non-susceptibility to at least one agent in all but two or fewer antimicrobial categories (i.e. bacterial isolates remain susceptible to only one or two categories) and PDR is defined as non-susceptibility to all agents in all antimicrobial categories. [4]. Detection of drug resistant organisms at the right time and within short period is one of the prime roles of a clinical microbiologist. Hence, the present study was undertaken to classify drug resistance among clinical isolates as MDR, XDR, PDR and to detect different mechanisms of resistance to beta-lactam antibiotics in gram positive and gram negative bacteria including MRSA, ESBL, AmpC and MBL by single plate sensitivity testing.

II. Materials and Methods:

2.1 Study population and clinical samples:

Study samples collected from both out-patients (OP) and in-patients (IP) departments, at Sri Muthukumaran Medical College Hospital and Research Institute, Chennai, from January 2014 to December 2014 were included in the study. Patients on antibiotic therapy for the past one month were excluded. The isolates were obtained from various clinical samples such as urine, pus, sputum, swabs, blood and other body fluids.

2.2 Antimicrobial sensitivity testing to detect MDR, XDR and PDR:

Antibiotic susceptibility testing was performed by Kirby-Bauer disc diffusion method as per CLSI guidelines [5]. As per Magiorakos AP, et al. specific categories of antibiotic discs were chosen against gram positive bacteria (Staphylococcus aureus) and for gram negative resistant bacteria to aid classification into MDR, XDR and PDR.

2.3 Single plate sensitivity testing to screen ESBL, AmpC and MBL bacteria:

All the Staphylococcus aureus isolates were screened for MRSA and GNBs were screened for ESBL, AmpC and MBL production by novel disc placement method. The antibiotic disc used to demonstrate these screening processes are listed in **Table - 1. [6, 7, 8].**

III. Result:

A total of 3176 clinical samples were included in this study, of which 472 (15%) and 756 (24%) were gram positive cocci (S. aureus) and gram negative bacilli respectively. Isolates showing resistance to at least one antimicrobial agent were considered as resistant isolates. Among the S. aureus isolates, 212 (45%) were MRSA (resistant to cefoxitin) and 260 (55%) were methicillin sensitive S. aureus (MSSA).

Among the 212 isolates of MRSA, 120 (57%) and 92 (43%) were in-patients (IP) and out-patients (OP) respectively. Among the 260 isolates of MSSA 75 (29%) and 185 (71%) were in-patients (IP) and out-patients (OP) respectively. The urinary tract was the most common site of infection with MRSA and MSSA isolates followed by wound, respiratory tract and various other sites. The antibiotic sensitivity pattern was evaluated for all S. aureus isolates from various classes of antibiotics to classify them into MDR, XDR and PDR. Highest number of isolates exhibited resistance to erythrocmycin (74%) followed by ciprofloxacin (55%), gentamicin (32%), tetracycline (25%), doxycycline (20%), rifampicin (14%), and chloramphenicol (13%). The highest sensitivity was observed to tigecycline 467 (99%) followed by linezolid 467 (99%), vancomycin 464, (98.3%), chloramphenicol (87%), rifampicin (86%) and other groups. As per Magiorakos AP, et al. all the MRSA isolates were considered as MDR-MRSA because resistance to cefoxitin predicts non-susceptibility to all categories of beta-lactam antimicrobials. Among 472 S. aureus isolates, 370 (78%) were MDR, while 79 (17%) and 0% were XDR and PDR respectively, and 23 (5%) isolates were resistant to less than three categories of antimicrobial agents (No MDR, XDR and PDR). Thus, the percentage of MDR was high among clinical isolates of S. aureus.

Among the 756 isolates of GNB 320 (42%) and 436 (58%) were in-patients (IP) and out-patients (OP) respectively. The urinary tract 548 (72%) was the most common site of infection with GNB isolates followed by wound, respiratory tract and various other sites. The antibiotic sensitivity pattern was evaluated for all the GNB isolates (Escherichia coli, Klebsiella species, Pseudomonas aeruginosa, Acinectobacter species, Enterobacter species, Citrobacter species, Morganella morgani and Proteus mirabilis) form various classes of antibiotics to classify them into MDR, XDR and PDR. Highest number of isolates exhibited resistance to ceftazidime (89%) followed by cefazolin (83%), doxycyline (79.2%), ciprofloxacin (75.4%), cefepime (72%), cefoxitin (65%), aztreonam (54%), chloramphenicol (54%), amikacin (27.3%) and piperacillin-tazobactam (20%). The highest sensitivity was observed to tigecycline (99.8%) followed by colistin (99.8%), polymyxin-b (99.8%), imipenem (99.3%), piperacillin-tazobactam (80%), amikacin (73%) and other groups. Among the GNB isolates 573 (76%) were MDR, while 181 (23.8%) and 2 (0.2%) were XDR and PDR respectively. Thus, the percentage of MDR was high among clinical isolates of gram negative bacteria. All the 756 GNB resistant isolates were screened for ESBL, AmpC and MBL production. Among the MDR isolates of GNB, 367 (64%) were ESBL producers, 47 (8%) were AmpC producers and coexistence of both ESBL and AmpC was found in 28 (5%). Amongst the XDR isolates, 89 (49%) were ESBL and 38 (21%) were AmpC producers, 3 (2%) were MBL producers and 23 (13%) were both ESBL and AmpC positive. In PDR isolates, 2 (0.2%) were MBL producers. Over all distribution of MDR, XDR, PDR and ESBL, AmpC, MBL in garm negative isolates are represented in **Table – 2 and Figure – 1**.

IV. Discussion

Infections caused by drug resistant bacteria lead to substantial morbidity and mortality, as well as high healthcare costs. This situation has been exacerbated by the rising incidence of strains that are less susceptible to a variety of antibiotics, making treatment of these infections more difficult [**9**]. The occurrence of co-resistance not only limits the therapeutic options but also poses a challenge for microbiology laboratories to identify them. The detection of drug resistant patterns and coexistence of various β-lactamases singly or in combinations is essential for enhanced infection control and effective antimicrobial therapy [**8**]. In view of resistant organisms being reported worldwide, we designed the present study to detect MDR, XDR, PDR and to screen different mechanisms of resistance to beta lactam antibiotics in gram positive and gram negative bacteria, including MRSA, ESBL, AmpC, and MBL by single plate sensitivity testing.

The cefoxitin disc diffusion method was found to be a reliable technique for MRSA detection. The emergence of MRSA has posed a major public health problem since the 1960s and it has become endemic in hospitals and intensive care units around the world and causes substantial morbidity and mortality [**10**]. For the first time, we have classified the S. aureus isolates into MDR, XDR and PDR. Notably, 370 (78%) and 79 (17%) out of the 472 isolates were found to be MDR and XDR respectively, with no PDR isolate. Following the spread of MRSA, glycopeptides and glycyclines such as vancomycin and tigecycline had become the mainstay of treatment for MRSA infections. Though emergence of vancomycin resistant S. aureus (VRSA) and vancomycin intermediate S. aureus (VISA) strains have been reported worldwide, in this study, 464 (98.3%) and 467 (99%) were sensitive to vancomycin and tigecycline respectively.

We also classified all the gram negative isolates as MDR, XDR and PDR according to guidelines recently published by an international expert panel [**4**]. To achieve this, we tested representative antimicrobials from different classes in our laboratory to determine non susceptibility within each class. With these caveats, these data show that, although the level was reduced, tigecycline retained good activity against MDR and XDR strains. Overall, this study was performed from January to December 2014, and we observed 76% of MDR, 23.8% XDR and 0.2% PDR in gram negative isolates. **As per Farrell DJ et al. 2013**, piperacillin-tazobactam activity was most compromised against the XDR Enterobacteriaceae and retained clear activity against many MDR and XDR strains. In our study, a similar kind of result was observed, followed by ceftazidime-clavulanic acid other than the higher antibiotic such as tigecycline and polymyxins. Many studies have described an ongoing outbreak of infection and colonization with PDR producing gram-negative organisms that are not susceptible to almost all widely used antibiotics [**12, 13**]. In this study we observed 2 (0.2%) PDR isolate resistance to tigecycline and polymyxins.

A very simple method (single plate sensitivity test) of placement of discs was used in this study to detect various β-lactamases. Imipenem disc in the center and cefoxitin disc acts as an inducer. Side by side placement of ceftazidime and ceftazidime + clavulanic acid disc around imipenem shows the ESBL-producers as ESBLs are inhibited by clavulanic acid. The blunting of zone of inhibition of ceftazidime discs toward inducers indicates the presence of inducible β-lactamases. Resistance to cefoxitin and cefepime sensitivity indicates constitutive production of AmpC β-lactamase. Decrease zone size of imipenem indicates presence of MBL. Resistance to cefoxitin, blunting of zone toward inducer, an increase of zone size with addition of inhibitor (ceftazidime + clavulanic acid and imipenem + EDTA) by 5 mm or more indicate multiple mechanisms involved [**6, 7, 8**]. In this study, highest numbers were ESBL, followed by AmpC in both MDR and XDR isolates. Three and two isolates were MBL producers in XDR and PDR respectively. Though the number appears to be small, it sounds an alarm for existence of pathogens likely to be highly resistant and expressions of various β-lactamases either singly or in combination.

Appling these definitions for MDR, XDR and PDR worldwide would allow comparability of data and promote better comprehension of the problem of highly antimicrobial resistant bacteria. These observations, compiled with previous literature show that antibiotic resistance is still on the rise. The high rates of MDR and XDR emergence have to be controlled before it shifts to PDR. Newer antibiotics have to be introduced and strict guidelines for prescription and usage need to be implemented.

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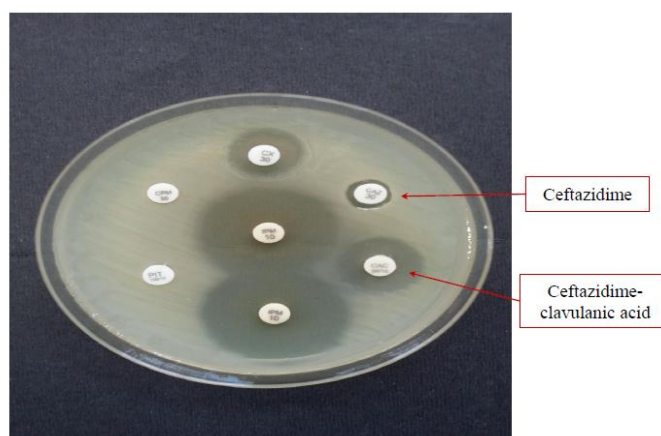
Figure – 1a: ESBL producer: The test isolate shows a zone Ceftazidime-clavulanic acid (CAC) sensitive > 5 mm compared to that of Ceftazidime (CAZ) disc.
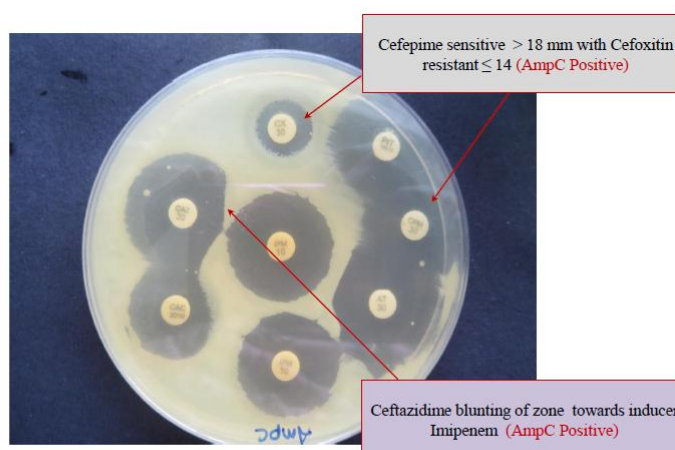

Figure – 1b: AmpC producer: The blunting of zone of inhibition of Ceftazidime (CAZ) discs toward inducers (IPM). Cefoxitin (CN) resistant ≤ 14 mm with Cefepime (Cpm) sensitive ≥18 mm.
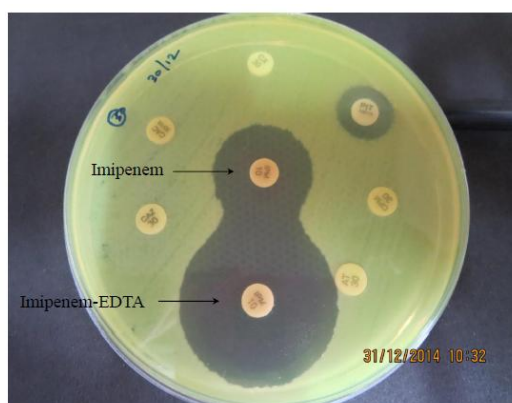
Figure – 1c: Detection of MBL producer by use of EDTA. The test isolate shows a zone diameter of ≥ 7 mm around the Imipenem-EDTA disc compared to that of the Imipenem disc.
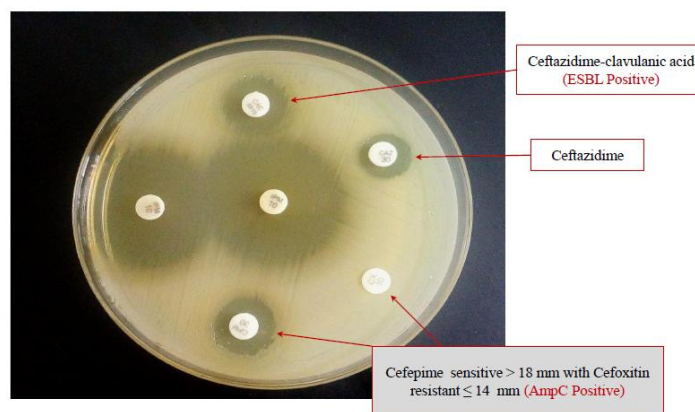

Figure – 1d: Coexistence of both ESBL and AmpC

Table 1: Screening of ESBL, AmpC, MBL and MRSA:

Resistance	Antibiotic used	Interpretation
MRSA	Cefoxitin (30µg)	Resistant
ESBL	Ceftazidime (30µg) and Ceftazidime-clavulanic acid (30 µg/10 µg)	Difference of ≥ 5mm
AmpC	Cefoxitin (30µg), Cefepime (30µg) or Imipenem (30µg)	1. Inducible β-lactamases (The blunting of zone of inhibition of Ceftazidime discs toward inducers). 2. Derepressed mutants of AmpC β-lactamase (Cefoxitin resistant ≤ 14 mm with Cefepime sensitive ≥18 mm).
MBL	Imipenem (10µg), Imipenem with 0.5 M EDTA.	Difference of ≥ 7mm

Table 2: Distribution of MDR, XDR, PDR and ESBL, AmpC, MBL in

Organisms (No.)	MDR – 573 (76%)				XDR - 181 (23.8%)				PDR - 2 (0.2%)		
	ESBL	AmpC	MBL	ESBL+ AmpC	ESBL	AmpC	MBL	ESBL+ AmpC	ESBL	AmpC	MBL
	367 (64%)	47 (8%)	-	28 (5%)	89 (49%)	38 (21%)	3 (2%)	23 (13%)	-	-	2 (0.2%)
Escherichia coli	167	8	-	12	27	11	-	8	-	-	-
Klebsiella species	95	10	-	8	16	7	-	11	-	-	-
Pseudomonas aeruginosa	22	10	-	-	15	7	3	-	-	-	2
Acinectobacter species	27	12	-	2	6	2	-	2	-	-	-
Enterobactor species	18	4	-	3	7	4	-	2	-	-	-
Citrobacter species	21	-	-	2	6	3	-	1	-	-	-
Morganella morgani	6	-	-	1	5	-	-	-	-	-	-
Proteus mirabilis	11	3	-	-	7	4	-	-	-	-	-

Note: Gram negative isolates (756)