

## Systematic Review

### Cefoxitin and oxacillin resistance conundrums

#### ABSTRACT

Recently, *mecA*-negative *Staphylococcus aureus* strains with decreased susceptibility to oxacillin and cefoxitin have been sporadically reported worldwide. They are called as borderline oxacillin resistant *Staphylococcus aureus* (BORSA). Almost, more than 30% of such strains are often misinterpreted and reported as MRSA (methicillin resistant *Staphylococcus aureus*) due to the hyperproduction of beta-lactamase enzyme and other reasons which results in invitro reduced susceptibility to oxacillin as well as cefoxitin. Similar phenomenon is quite common in other staphylococci, micrococci and macrococci. In order to identify beta-lactamase hyperproducing staphylococci and other gram-positive cocci and to avoid false reporting as methicillin resistant, a one-year prospective study was conducted in Deccan college of medical sciences, Hyderabad, Telangana state, India in 2023. During one year duration, 5630 inpatient (IP) and 823 outpatient (OPD) samples accounting for total 6453 cultures were received in microbiology laboratory. Out of this, 100 cultures of different samples showing pure growth of gram-positive cocci suggestive of staphylococci, micrococci and other Gram-positive cocci were included in the present study and tested against different antibiotics panel as per CLSI guidelines (Clinical and Laboratory Standards Institute.) Inclusion of amoxyclav disc to the antibiotics test panel as per recommendation by the special phenotypic methods for the detection of antibacterial resistance in the manual of clinical microbiology was helpful to detect the beta-lactamase hyperproducers (BHP) and helped to report correct identification of the organisms and curtailed the mismanagement of more than 95% of the patients. Therefore, I do recommend to differentiate borderline from true methicillin resistant strains to streamline the antibiotic therapy and hence further avoidance of selection of the resistant strains.

**KEYWORDS:** Gram positive cocci, staphylococci, susceptibility to cefoxitin, oxacillin, amoxyclav, BORSA interpretation.

## INTRODUCTION

*Staphylococcus aureus* specially MRSA, is a major pathogen in the hospitals and community, mainly responsible for the skin lesions, pneumonia, bacteremia, endocarditis and toxic shock syndrome and hospital acquired infections. MRSA strains carry penicillin-binding protein 2a (PBP2a), a low-affinity PBP encoded by *mecA* gene and homologues. Apart from *mecA*, methicillin resistance is also associated with  $\beta$ -lactamase enzyme hyperproduction, multiple unlinked mutations in native *pbp* genes that reduce the affinity of PBPs for  $\beta$ (beta)lactams as well as mutations in the *pbp4* promoter and/or in genes *gdpP*[phosphodiesterase c-di-AMP regulator] and *yjbH*(disulfide stress effector) that lead to PBP4 overproduction.<sup>1,2</sup>

Staphylococci with cefoxitin (CX) and/or oxacillin (OX) resistance but without *mec* determinants (methicillin-resistant lacking *mec*[MRLM] strains) have been reported from 1980s to the recent years. This phenotype is caused by the hyperproduction of  $\beta$  lactamase enzyme, encoded by the *blaZ* gene that partially hydrolyzes semisynthetic beta-lactamase resistant penicillins, oxacillin and cepheems and results in reduced invitro susceptibility and often misinterpreted as methicillin resistant. These *mec* A negative,  $\beta$ -lactamase hyperproducers (**BHPs**) recover full susceptibility to  $\beta$  lactams in the presence of the  $\beta$  lactamase inhibitors. Though the mechanisms of resistance to oxacillin in borderline resistant staphylococcal strains have not been clarified yet, likely candidates are hyperproduction of  $\beta$ -lactamase, overexpression and/or mutations in PBP4 or production of a novel oxacillin hydrolyzing  $\beta$ - lactamase.<sup>1-14</sup> Besides this, it also occurs because of amino acid substitutions, Gln629 to RPro, in the transpeptidase domain belonging to sequence type 25 (ST) lineage as well as single amino acid substitution, Asp606 to RVal, in the transpeptidase domain in ST1 and likewise in other lineages as well.<sup>13</sup> BHP isolates are usually named as borderline oxacillin-resistant *Staphylococcus aureus* (BORSA), while isolates with resistance due to mutations or production of modified intrinsic PBPs with altered affinity for drug are named as modified *Staphylococcus aureus* (MOD-SA)<sup>13</sup> however, few authors have used these terms interchangeably. BORSA phenotype can be detected in 1.2–12.5%, 10.73% of clinical *Staphylococcus aureus* isolates.<sup>2,5,6,7</sup> whereas as per one study of 500 healthy children, staphylococcal strains with the borderline phenotype were isolated from more than 5% of the subjects.<sup>6,15,16</sup>

In order to detect the hyperproducers of the  $\beta$ -lactamase BORSA strains 'masquerading' as MRSA and for better management of the patients, a prospective study of one year duration with inclusion of 100 strains of Gram-positive cocci was undertaken at Deccan college of medical sciences, Hyderabad, Telangana state, India.

## MATERIAL AND METHODS

This research study was conducted from 11/2/23 to 31/12/23 at Deccan college of medical sciences, Hyderabad, Telangana state, India. I did include the pilot study samples from 01/01/23 also to this study which constituted as one-year prospective study (01.01/23 to 31/12/23.) Ethical clearance was applied on 08/05/2023 and received 20/02/2024.

During one year duration, total 5630 IP and 823 OP samples accounting for grand total of 6453 were received in the microbiology laboratory. Out of this, total 100 samples such as exudates, urine and blood etc received for culture and sensitivity at Deccan **college of medical sciences**, Hyderabad, India from IP (inpatient) and OPD (outpatient department) sections, suggestive of monomicrobial infections were included in the prospective study. The history, diagnosis, compliance to the current therapy of all IP patients and follow up were inquired telephonically. Histories of OPD patients were not available.

All exudates and blood samples were inoculated on blood agar, MacConkey agar and nutrient agar and urine specimens on MacConkey agar, CLED (cysteine lactose electrolyte deficient agar) and UTI chrome agar (Himedia) and incubated for 24 hours under aerobic condition at 37 °C except blood cultures for 7 days. Later, direct smears from the clinical samples were stained with Gram stain as per the requests of the clinicians. All suspected positive cultures of monomicrobial origin displaying growth of Gram-positive cocci isolates were identified by cultural characteristics, Gram stain and conventional biochemical tests like catalase, slide and tube coagulase test, novobiocin susceptibility etc up to the species level.<sup>11</sup> Susceptibility was done by Kirby Baur (KB) disc diffusion method on 90mm Muller Hinton agar plates as per CLSI guidelines.<sup>12</sup>

Beta lactamase mediated resistance (MOD-SA/BORSA) is differentiated from classic (mecA positive) type of resistance by the special phenotypic methods for detection of antibacterial resistance recommended as per the manual of clinical microbiology with the inclusion of beta lactamase inhibitor like clavulanic acid to oxacillin MIC method, which lowers MIC by two dilutions or more.<sup>13</sup> As MIC method was not available, amoxycylav disc (20/10 µg-Himedia, India) along with cefoxitin (30 µg-Himedia) and oxacillin (1 µg-Himedia) was included in the antibiotic testing panel by Kirby Baur disc diffusion method to check the hyperproducers of *Staphylococcus spp* and other Gram positive cocci isolates in order to differentiate borderline from true methicillin resistance.<sup>4-7</sup> They were also tested against vancomycin, teicoplanin, linezolid, ciprofloxacin, levofloxacin, gentamicin, amikacin, nitrofurantoin (for urine samples only), erythromycin, clindamycin, cotrimoxazole, doxycycline, imipenem, ampicillin and azithromycin for all samples. The susceptibilities of the identified organisms to cefoxitin, oxacillin and other antibiotics was documented as per the CLSI guidelines.<sup>12</sup> The zone size of inhibition of amoxycylav was interpreted for staphylococci and other Gram-positive cocci.<sup>14</sup>

The final antimicrobial susceptibility results were informed to the respective clinicians of the wards to check for the clinical correlation and compliance to the present treatment and the follow up.

### **Results:**

The present one-year prospective study analysed total 100 samples, out of which 10 were from OPD and 90 from IP and included 33 females and 67 men, ranging from

neonates to old people. This work dealt with total 60 blood, 4 sputum, 2 endotracheal aspirations, 18 pus, 6 wound swabs, 9 urine and 1 throat swab.

Standard guidelines were followed for all sample processing such as Gram stain, culture and the identification of gram-positive cocci like staphylococci, micrococci etc with the manual conventional biochemical tests and antimicrobial susceptibility test as per CLSI guidelines.<sup>11-13</sup> 92 were identified as *Staphylococcus aureus*, 5 as *Staphylococcus epidermidis* and 3 as *Micrococcus luteus*. Most of staphylococci (77.6%) formed 1 to 2 mm white colonies and were nonhemolytic except 39 (42.3%) were beta hemolytic whereas 19 (20.6%) and 2 (2.1%) of cultures grew yellow and orange pigmented strains respectively and only one strain formed big, nonhemolytic white colony measuring from 3 to 4 mm. Cultural characteristics of *Staphylococcus epidermidis* and *Micrococcus luteus* did not reveal any significant differences in the usual colony morphology. Gram stain and biochemical tests of all strains did not reveal any significant phenotypic differences in respect to their identification.

Most of the isolates were susceptible to most of the classes of antibiotics except 21%, 2%, 33%, 6%, 4%, 11%, 30% and 84% were resistant to cotrimoxazole, doxycycline, erythromycin and clindamycin, levofloxacin, gentamicin, ciprofloxacin, azithromycin and ampicillin respectively.

All gram-positive isolates (100 %) did display reduced zones of inhibition to ceftiofur, oxacillin depending on their identification<sup>13</sup> and susceptible zones ( $\geq 20$  mm for staphylococci and  $\geq 18$  mm for others<sup>14</sup>) or bigger zones for amoxiclav ranging from 23 to 28 mm as depicted in the figure 1. Zone diameters of staphylococci were used for interpretation of susceptibility to oxacillin for micrococci as they are not mentioned in CLSI and no other sources.<sup>14</sup> and ceftiofur susceptibility was interpreted as mentioned in susceptibility test methods: dilution and disk diffusion methods.<sup>14</sup>

The identified strains along with respective AST (antimicrobial susceptibility test) pattern were informed to the clinicians and were enquired about the variable diagnosis and current therapy. Majority of the IP patients (> 90%) who were on present therapy like ceftriaxone and piperacillin/tazobactam monotherapy and/or in combination with other classes of antibiotics, responded and improved well with the current therapy and discharged within 4 to 5 days without escalation or de-escalation on the follow up. Few challenging cases eventually improved with the treatment. Total 4 deaths occurred during this study. Histories of OPD patients were not available.

During this one-year academic analysis, total 5630 IP and 823 OP samples accounting for grand total 6453 were received in the microbiology laboratory. Lower colony count from urinary and endotracheal secretions cultures, polymicrobial infections suggestive of staphylococci, micrococci along with gram negative bacilli or *Candida spp* etc were not included in the study though they were checked for the hyperproducibility of the beta lactamase and revealed that approximately 37.2 % of the strains were methicillin sensitive and 62.8 % were borderline resistant. In the present study, all included isolates from the different 100 samples were identified as hyperproducers of beta lactamase (100%).

## DISCUSSION:

MRSA is a superbug responsible for the life devastating conditions hence warrants early identification and susceptibility results. Conversely, incorrect identification and reporting of BORSA strains as MRSA would be disastrous and could mislead the management of the patient and would levy unnecessary charges on the patient for the hospital stay, isolation, antibiotic therapy etc.

In the present scrutiny, BORSA strains were isolated different sites, from OPD as well as IP patients with variable diagnosis. Similarly, BORSA strains isolated from various sites such as skin, surgical wounds, respiratory samples, abscess and blood were implicated in health care associated and community-acquired infections in some documented studies. Apart from this, outbreaks of BORSA infections were seen as reported in two different dermatological units in Denmark.<sup>7,17-22</sup> On the contrary, there was not even a single incidence of outbreak during the present research.

*Staphylococcus aureus* that demonstrates decreased susceptibility to ceftazidime and oxacillin or is susceptible to increased minimal inhibitory concentrations (MICs) of other beta-lactams, but that does not carry the *mec*-type genes, has been detected. Such isolates are commonly referred as borderline oxacillin-resistant *Staphylococcus aureus* (BORSA.) There is currently no consensus on the terminology. Some authors prefer the general term, methicillin-resistant lacking *mec* gene (MRLM) and other authors mention as BORSA, in a narrow sense for beta-lactamase hyperproducers (BHPs) and modified *Staphylococcus aureus* (MODSA) for isolates with resistance due to mutations or production of modified intrinsic PBPs with altered affinity for drug.<sup>2,13</sup> Staphylococcal beta-lactamases (BlaZ) are serologically classified into types A to D and type C BlaZ from BORSA hydrolyzes oxacillin faster than MSSA. In MODSA, mutations in the *pbp* gene, the *pbp4* promoter region and *gdpP* (c-di-AMP regulator) and *yjbH* (disulfide stress effector) genes are known to increase resistance to oxacillin and other beta-lactams in the absence of *mec*-type genes.<sup>2</sup> Sometimes, borderline resistance could be because of the presence of the novel methicillinase enzyme.<sup>13</sup>

Beta lactamase mediated resistance (MOD-SA/BORSA) is differentiated from classic (*mecA* positive) type of resistance by the special phenotypic methods for detection of antibacterial resistance recommended as per the manual of clinical microbiology with the inclusion of beta-lactamase inhibitor like clavulanic acid to oxacillin MIC method, which lowers MIC by two dilutions or more<sup>13</sup>. Due to unavailability of oxacillin MIC method, amoxycyclav disc (20/10 µg-Himedia, India) along with ceftazidime (30 µg-Himedia) and oxacillin (1 µg-Himedia) was included in the antibiotic testing panel to check the hyperproducers of *Staphylococcus spp* and other Gram positive cocci isolates in order to differentiate borderline from true methicillin resistance<sup>4-7</sup>. All gram-positive isolates (100 %) did display zones ( $\geq 20$  mm for staphylococci and others  $\geq 18$  mm<sup>14</sup>) or bigger zones for amoxycyclav ranging from 23 to 28 mm. Susceptible zone size of  $\geq 25$  mm for amoxycyclav<sup>7</sup> is mentioned by France study.

Another method to confirm the absence of beta-lactamase and presence of beta-lactamase hyperproduction as the mechanism responsible for the BORSA

phenotype, the activity of clavulanic acid in combination with two beta-lactam antimicrobial agents (cefotaxime and ceftazidime) was done by Canadian study. It is demonstrated by disc diffusion testing by using cefotaxime (30 µg), cefotaxime-clavulanic acid (30 and 10 µg respectively), ceftazidime (30 µg) and ceftazidime-clavulanic acid (30 and 10 µg respectively) discs.<sup>15,23</sup> The presence of clavulanic acid should result in a significant change (>5 mm) in cefotaxime or ceftazidime zone sizes or significant decrease in the MIC by  $\geq 2$  doubling dilutions after the addition of beta-lactamase inhibitor.<sup>15,24</sup>

In this study, all Gram-positive isolates (100 %) did display reduced zones diameters to cefoxitin and oxacillin depending on the identification and susceptible zone of inhibition of amoxicillin and clavulanic acid combination (amoxycrav.) As per one study, majority of the isolates 72.2 % (26/36) tested positive for  $\beta$ -lactamase production while only 11/26 (42.3%) were  $\beta$ -lactamase hyperproducers whereas Nigerian study revealed 63% of hyperproducers of beta-lactamase.<sup>1, 4</sup>

In this one-year analysis, total 5630 IP and 823 OP samples accounting for the grand total of 6453 were received in the microbiology laboratory. Urinary and endotracheal secretions cultures with lower colony count, polymicrobial infections suggestive of staphylococci, micrococci along with gram negative bacilli, *Candida spp* etc were not included in study though they were checked for hyper-producibility of beta-lactamase and revealed that overall 37.2 % of Gram positive strains were methicillin sensitive and 62.8 % did showcase borderline oxacillin resistance, an indicator of high percentage of oxacillin resistance. So from my point of view, the detection of beta-lactamase is utmost importance in the management of the patients and to curb unnecessary lengthy hospital stay and expenditure.

Belgium study revealed that 32 (10.73%) isolates of *Staphylococcus aureus* showed resistance to oxacillin (OX) and cefoxitin (CX), with individual representation of resistance of 25 % and 18.75 % respectively and combined resistance of 56.25% and all were *mecA*, *mecB*, *mecC* and PBP2a negative.<sup>5</sup> All isolates were recovered mostly from nasal/skin screening samples, wound/skin infection and ear, nose and throat displaying isolation percentage of 56.25, 25 and 9.6 respectively and 3.12 % from blood, urine and unknown samples each. Similar school of thought had reported recovery of BORSA strains from different sites.<sup>5</sup> Nigerian study also stated about the higher percentage of beta-lactamase producing cells that showcased resistance to cloxacillin and flucloxacillin were 71% and 57% respectively. These BORSA strains showed resistance to penicillinase resistant penicillin (PRP) because of the hyperproduction of beta-lactamase which can easily be counterbalanced with the inclusion of enzyme inhibitors such as clavulanic acid or sulbactam. This distinguishes BORSA from MRSA strains, where beta-lactamase inhibitors contribute to a decrease in the penicillin MIC but do not alter the MIC of PRPs, even at higher concentrations.<sup>6</sup> Conversely, 14% of BORSA strains were resistance to amoxicillin and clavulanic acid combination, may be highlighting another mechanism for resistance to OX, CX and amoxycrav apart from *mec* gene.<sup>4</sup> In the present workup, none of the BORSA strain was resistant to amoxycrav.

Belgium study documented that most of the isolates, 81% carried an active  $\beta$  lactamase (*blaZ*) but only 37.5% were PDDT (penicillin disk diffusion test) positive and BHPs and displayed different mutations. 10.7% of such isolates were associated

with 13 lineages, with a predominance of clonal complex 25 (CC25) (21.8%). CC25 has been described as the most frequent lineage with the MRLM phenotype in Canada but this clone is rarely found in Belgian hospitals. MRLM strains belonging to CC1, CC8, CC15, and CC45 in clinical settings and strains belonging to CC45 and CC398 from livestock were described previously.<sup>5</sup> The remaining 62.5% isolates (including 14 *blaZ*-positive/PDDT-positive isolates and 6 *blaZ*-negative/PDDT-negative isolates) were non-BHPs and had diverse mutations. Genotyping and clonal typing were not done during present analysis.

Not only the increased production of beta-lactamase enzyme, the borderline resistant phenotype has been attributed to other mechanisms also, i.e., the production of an inducible, plasmid-mediated methicillinase or different modifications in the PBP genes due to spontaneous amino acid (AA) substitutions in the transpeptidase domain<sup>7</sup> as well as in the transglycosylase domains. Certain AA substitutions affecting  $\beta$  lactam resistance (A405V and Q629P in PBP2) are described previously. Some AA substitutions (Y336C, T371I, and H499Y in PBP1 and S364F in PBP3) were detected previously in MRLM strains from CC1, CC8, and CC15.<sup>5</sup>

Equivalently, Poland review article also mentioned about different possible determinants like modifications in PBP3 and/or PBP4 and the selective pressure of beta-lactam antibiotics responsible for BORSA phenotype. Such versions of BORSA strains constitute a particular threat to the epidemiological and therapeutic perspectives. To be distinguished from beta-lactamase-hyperproducing BORSA, they are referred to as modified *Staphylococcus aureus*. Similar to the MRSA strains, MODSA do not become susceptible to PRPs after the addition of a beta-lactamase inhibitor to oxacillin.<sup>6</sup> In contrast to this, many authors had used terms BORSA or MODSA interchangeably in many journal articles and even in reference textbooks.<sup>1-5,7-10,12,13</sup>

Russian study reviewed about mutations in c-di-AMP signal transduction pathways, chaperone-protease complexes (clpX, clpP), CW regulators (*vraRS* and *graRS*), mutations in *pbp1* and *pbp2* are recognized as the main mechanism of low-level oxacillin resistance.<sup>2</sup>

Susceptibility to cefoxitin is a good marker of staphylococcal sensitivity to PRPs and other beta-lactams. Unfortunately, this parameter cannot be considered as a marker of borderline methicillin resistance if it involves a different mechanism other than the presence of PBP2a (*mecA/C* gene). Borderline oxacillin resistant strains are better identified as methicillin-sensitive and methicillin-resistant based on the susceptibility to cefoxitin, oxacillin and amoxyclovan antibiotics. If only susceptibility to cefoxitin is tested, a borderline strain may be misidentified as a methicillin resistant isolate. Therefore, it is recommended that the combination of three antibiotics should be tested against the organisms for the detection of the borderline resistant Gram-positive isolates in a laboratory setting.<sup>4-7,25,26</sup>

Recently, chromogenic media used for the detection of MRSA such as Select II (BioRad, USA), Colorex MRSA (E&O laboratories, UK), ChromID MRSA (bioMérieux, France) and MRSA Brilliance 2 (Oxoid, UK) had reported decreased specificity with more recovery of 50% of BORSA strains.<sup>6,29</sup> From my point of view, in the near future, majority of Gram positive cocci would be correctly identified as methicillin sensitive, and borderline resistant and only few would be labeled as

methicillin resistant due to inclusion of optimal phenotypic and genotypic methods. This is also proved by many retrospective analytical studies. As per one study, all recovered BORSA strains (1.2%) were negative for presence of PBP2a and mecA gene by PCR.<sup>7</sup>

Genotyping methods like PFGE, could one of the best way to discriminate between MSSA, BORSA and MRSA as documented by one of the group of studies. Many BORSA strains belonged to ST25 lineage.<sup>6,30</sup> Besides this, other promising studies involving various genotyping methods like PFGE, MLST and spa typing had demonstrated promising results in detection of high degree of strain relatedness in BORSA and may be indicative of belongingness to single clone and/or lineage and ultimately, helps in exact reporting of the results.

Borderline strains are treated by cephalosporins, beta-lactamase inhibitors, aminoglycosides, fluoroquinolones etc. In the present academic study, most of the cases were treated by ceftriaxone, piperacillin/tazobactam, clindamycin, doxycycline and imipenem. Similar treatment choices are also mentioned by equivalent studies.<sup>7</sup> Sometimes, borderline strains could multidrug resistant. In the present study, few challenging cases were seen. Culture of blood and urine sample of one adult male patient diagnosed with pulmonary tuberculosis grew BORSA and MRSA respectively and responded well to linezolid and piperacillin/tazobactam combination therapy. Another one identical case of mecA negative and PBP2a negative endocarditis with oxacillin MIC 12 µg/ml (E-test) and negative nitrocefin confirmed by the lack of a significant increase in the growth inhibition zones around cefotaxime-clavulanic acid and ceftazidime, recorded therapeutic success with shifting the treatment from oxacillin to vancomycin.<sup>6,25</sup> Similarly, one death of the hospitalized patient with the diagnosis of septic shock occurred within 48 hours before positivity of the culture, may be due in vivo transition of borderline to methicillin resistance in the present study. On contrary, one patient admitted with diagnosis of eczema with burning micturition, showed scanty growth of BORSA with the colony count of 1000 CFU/ml in the urine sample and improved just by drinking plenty of water and discharged without any antibiotic therapy in the present study.

Most of *Staphylococcus aureus* strains do display pathogenic characteristics in the culture morphology like coagulase production, presence of beta hemolysis, yellow pigment, mannitol fermentation etc. and may correlate with the invasiveness, complications and death.<sup>11</sup> In the present work, 2 beta hemolytic without pigment, 1 golden yellow pigmented as well as beta hemolytic and 1 nonhaemolytic and nonpigmented BORSA strains were isolated from different patients and responded well to the current therapy but death of these four patients occurred eventually in the due course of the disease during hospital stay, may be because of end stage diseases associated with comorbid conditions or in vivo transition of BORSA to MRSA. So, from my point of view, neither the cultural characteristics like golden yellow pigment nor other vital features like coagulase production, beta hemolysis etc were associated with the negligible mortality (<5%) although it may be attributable to long standing diseases associated with comorbidities as per histories of the patients.

## Conclusion:

*mecA* negative *Staphylococcus aureus* strains with the decreased susceptibility to oxacillin have been sporadically reported worldwide and are responsible for community as well as hospital acquired infections. They have been called as borderline oxacillin-resistant *Staphylococcus aureus* (BORSA).  $\beta$ -lactamase enzyme is encoded by the *blaZ* gene, is categorized as a class A  $\beta$ -lactamase (penicillinase) which shows low hydrolytic activity against oxacillin, cepheems and carbapenems.

Many clinical microbiology laboratories use only the cefoxitin test for detection of oxacillin resistance in *Staphylococcus spp.* Indeed, the cefoxitin test is a marker of resistance to oxacillin by acquisition of the *mecA* gene but it is unable to detect borderline resistant strains. In our study, cefoxitin, oxacillin and amoxycylav were used to detect such strains. Similar suggestion are quoted by many school of thoughts, especially for *Staphylococcus aureus* and other staphylococci.<sup>4-7</sup> I do recommend to use combination of these discs, in my words "**Triad Panel**" to identify borderline resistance in all staphylococci and likewise in micrococci, macrococci and other gram-positive cocci and represented as well with better sensitivity and specificity in other studies.<sup>4-7</sup> In the present study, all isolated clinical strains were borderline resistant (BHPs) and responded well to beta lactams. It very crucial to identify it in order to streamline the treatment and would avoid the mismanagement of the patients and would not enforce unnecessary hospital charges on patient for isolation and selection of last resort of drugs like vancomycin, linezolid etc for clinicians. It also decreases the hospital stay and avoids selection of antimicrobial resistance determinants and simultaneously emergence of hospital acquired infections.

In present study, none of the borderline strain was resistant to betalactamase inhibitor like amoxycylav though few studies had mentioned about it.<sup>4</sup> From my point of view, the day is not far away from us, inclusion of optimal phenotypic and genotypic methods would be necessary for interpretation of results. In the future, most of the methicillin resistant strains would be identified as borderline resistant by retrospective studies. **Trilogy** of oxacillin with cefoxitin and amoxycylav disc diffusion susceptibility testing, MIC, amino acid sequencing, evaluation of oxacillin hydrolysis activity, PCR, PFGE, MLST and spa are the different methods to identify the BORSA strains.<sup>3-7,30</sup>

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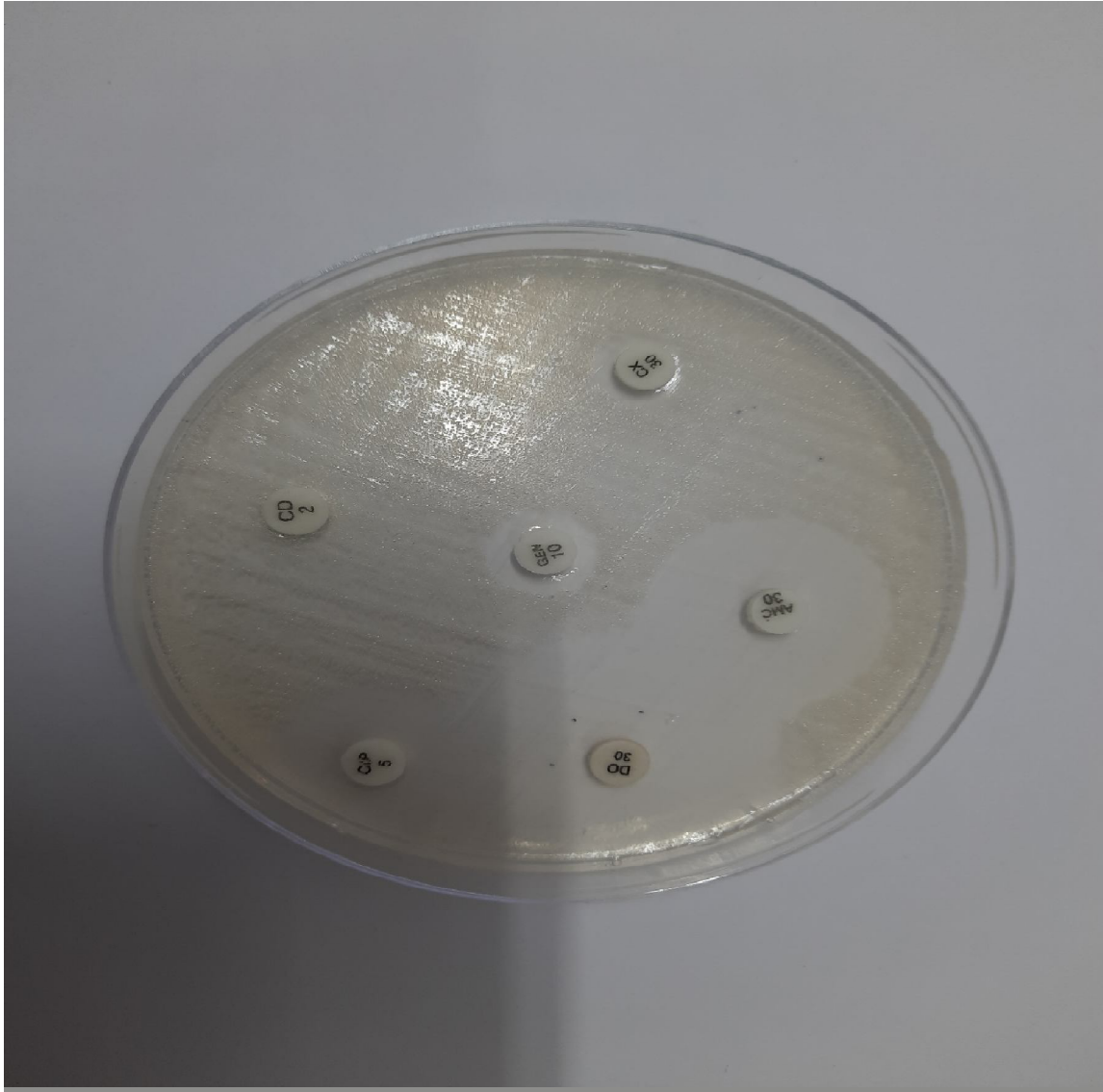
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**FIGURE 1 SHOWING REDUCED SUSCEPTIBILITY TO CEFOXITIN (CX) AND SUCCEPTIBLE ZONE OF AMOXYCLAV (AMC)**

UNDER PEER REVIEW