Systematic Review

Cefoxitin and oxacillin resistance conundrums: A review

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 β-lactamase enzymehyperproduction, multiple unlinked mutations in native *pbp genes* that reduce the affinity of PBPs for β(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.[2]

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 therecent years. This phenotype is caused by the hyperproduction of β lactamaseenzyme,encoded by the blaZ gene that partially hydrolyzes semisynthetic beta-lactamase resistant penicillins, oxacillin andcephems and results in reduced invitro susceptibility and often misinterpreted as methicillin resistant. Thesemec A negative, β-lactamase hyperproducers **(BHPs**)recover full susceptibility to β lactams in the presence of the β lactamase inhibitors. Though the mechanisms of resistance to oxacillin in borderline resistant staphylococcal strains have not been clarified yet, likely candidates are hyperproduction of β-lactamase, overexpression and/or mutations in PBP4 or production of a novel oxacillin hydrolyzing β- lactamase.[1-14] 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.[13]BHP isolates are usually named as borderline oxacillin-resistant *Staphylococcus aureus* (BORSA) , while isolates with resistance due to mutationsor production of modified intrinsic PBPs with altered affinity for drugare named as modified *Staphylococcus aureus* (MOD-SA)[13] however, few authors have used these terms interchangeably.BORSA phenotype can be detected in1.2–12.5%,10.73% of clinical*Staphylococcus* aureus isolates.[2,5,6,7] whereas as per one study of 500 healthy children,staphylococcal strains with the borderline phenotype were isolated from more than 5% of the subjects.[6,15,16]

In order to detect the hyperproducersof the β-lactamaseBORSA strains ‘masquerading’ as MRSA and for better management of the patients, a prospective study of one year durationwith 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.) Ethicalclearance 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, total100 samplessuch as exudates, urine and bloodetc 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 therapyof all IP patients and follow upwere inquiredtelephonically. 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 ^{0} 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-positivecoccalisolates 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.[11]Susceptibility was done by Kirby Baur (KB) disc diffusion method on 90mm Muller Hinton agar plates as per CLSI guidelines.[12]

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 betalactamase inhibitor like clavulanic acid to oxacillin MICmethod, which lowers MIC by two dilutions or more. [13]As MIC method was not available,amoxyclav disc (20/10 µg-Himedia,India) along with **c**efoxitin (30µg-Himedia) and oxacillin(1 µgHimedia)was included in the antibiotic testing panel by Kiry Baur disc diffusion method to check the hyperproducers of *Staphylococcus spp*andother Gram positive coccalisolates in order to differentiate borderline from true methicillin resistance**.**[4-7]They were also tested against vancomycin, teicoplanin, linezolid, ciprofloxacin, levofloxacin, gentamicin,amikacin,nitrofurantoin (for urine samples only), erythromycin, clindamycin,cotrimoxazole,doxycycline,imipenem,ampicillin and azithromycinfor all samples. The susceptibilities of the identified organisms to cefoxitin, oxacillin and other antibiotics was documented as per the CLSI guidelines.[12]The zone size of inhibition of amoxyclav was interpretated for staphylococci andother Gram-positive cocci.[14]

Thefinal 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 presentone-year prospective studyanalysed total 100 samples, out of which 10 were from OPD and 90 from IP and included 33 females and 67men, 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 forall samplesprocessing such as Gram stain, cultureand the identification of gram-positive cocci like staphylococci, micrococci etc with the manual conventional biochemical tests and antimicrobial susceptibility test as per CLSI guidelines.[11-13]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 nonhemolyticexcept 39(42.3%)were beta hemolytic whereas 19(20.6%) and 2(2.1%)of cultures grew yellow and orange pigmented strains respectivelyand only one strain formedbig,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 theiridentification.

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 cefoxitin, oxacillin depending on their identification[13]and susceptible zones (≥ 20 mm for staphylococci and ≥ 18mm for others[14]) orbigger zones foramoxyclavranging from 23 to 28 mm as depicted in the figure 1. Zonediameters of staphylococci were used for interpretation of susceptibility to oxacillin for micrococci as they are not mentioned in CLSI and no other sources.[14] andcefoxitin susceptibility was interpretated as mentioned in susceptibility test methods: dilution and disk diffusion methods.[14]

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/tazobactummonotherapy and/or in combination with other classes of antibiotics, responded and improved well with the current therapyand 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 bacillior*Candidaspp*etcwere not included in thestudy though they were checked for the hyperproducibilityof the betalactamaseand revealedthat approximately37.2 % of the strains were methicillin sensitiveand 62.8 % were borderline resistant.In the present study, all included isolates from the different 100 samples were identified as hyperproducers of betalactamase(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 forthe 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 varioussites such as skin, surgical wounds, respiratory samples, abscess and bloodwere 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. [7,17-22]On the contrary, there was not even a single incidence of outbreak during the present research.

Staphylococcus aureus that demonstrates decreased susceptibility to cefoxitin and oxacillin or is susceptible to increased minimal inhibitory concentrations (MICs) of other betalactams,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 lackingmec 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.[2,13]Staphylococcal beta-lactamases (BlaZ) are serologically classified into types A to D andtype C BlaZ from BORSA hydrolyzes oxacillin faster thanMSSA. In MODSA, mutations in the pbpgene,the pbp4 promoter regionand gdpP (c-di-AMP regulator)and yjbH (disulfide stress effector) genes are known toincrease resistance to oxacillin and other beta-lactams in the absence of mec-type genes.[2] Sometimes, borderline resistance could be because of the presence of the novelmethicillinase enzyme.[13]

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 betalactamase inhibitor like clavulanic acid to oxacillin MIC method, which lowers MIC by two dilutions or more[13].Due to unavailability of oxacillin MIC method,amocyclav disc (20/10 µg-Himedia, India) along with**c**efoxitin (30µg-Himedia) and oxacillin (1 µgHimedia) was included in the antibiotic testing panel to check the hyperproducers of *Staphylococcus spp* and other Gram positive coccal isolates in order to differentiate borderline from true methicillin resistance[4-7] All gram-positive isolates (100 %) did display zones (≥ 20 mm for staphylococci and others ≥ 18mm [14]) or bigger zones for amoxyclav ranging from 23 to 28 mm.Susceptible zone size of ≥ 25 mm for amoxyclav[7]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. [15,23] 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 ≥2 doubling dilutions after the addition of beta-lactamase inhibitor.[15,24]

In this study,all Gram-positiveisolates (100 %) did display reduced zones diameters to cefoxitin and oxacillin depending on the identification and susceptiblezone of inhibition of amoxicillin and clavulanic acid combination (amoxyclav.) As per one study, majority of the isolates 72.2 % (26/36) tested positive for β-lactamase production while only 11/26 (42.3%) were β-lactamase hyperproducers whereas Nigerian study revealed 63%of hyperproducers of betactamase. [1, 4]

In this one-yearanalysis, 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*etcwere not included in study though they were checked for hyper-producibilityof 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 betalactamase is utmost importance in the management of the patients and to curb unnecessary lengthy hospital stay and expenditure.

Belgium study revealed that32(10.73%) isolates of *Staphylococcusaureus* showed resistance to oxacillin(OX) and cefoxitin(CX),with individual representation ofresistance of 25 % and 18.75 % respectively and combined resistance of 56.25%and all were *mecA*, *mecB*, *mecC* and PBP2a negative[5] 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 hadreported recovery of BORSA strains from different sites.[5] Nigerian studyalso stated about the higherpercentage of beta-lactamase producing cells that showcased resistance to cloxacillin and flucloxacillin were 71% and 57% respectively. These BORSAstrains showed resistance topenicillinase resistant penicillin (PRP) because of the hyperproduction of beta-lactamasewhich can easily be counterbalanced with the inclusion of enzyme inhibitors such as clavulanic acidor 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.[6]Conversely,14% of BORSA strains were resistance to amoxicillin andclavulanic acidcombination, may be highlighting another mechanism for resistance to OX,CX and amoxyclav apart from mecgene.[4]In the present workup, none of the BORSA strain was resistant to amoxyclav.

Belgium study documented that most of the isolates, 81% carried an active β 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.[5]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[7] as well as in the transglycosylasedomains.Certain AA substitutions affecting β 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. [5]
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 threatto 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 became susceptible to PRPs after the addition of a beta-lactamase inhibitor to oxacillin.[6] In contrast to this, many authors had used terms BORSA or MODSA interchangeably in many journal articles and even in reference textbooks.[1-5,7-10,12,13]

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 pbp2are recognized as the main mechanism of low-level oxacillin resistance.[2]

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 amoxyclavantibiotics.If only susceptibility to cefoxitin is tested, aborderline strain may be misidentified as an methicillin resistant isolate.Therefore, it is recommended that the combination of three antibiotics should be tested against the organisms forthe detection of the borderline resistant Gram-positive isolates in a laboratory setting.[4-7,25,26.]
Recently, chromogenic media used for the detection of MRSA such as Select II (BioRad, USA), Colorex MRSA (E&O laboratories, UK), ChromID MRSA (bioMerieux, France) and MRSA Brilliance 2 (Oxoid, UK) had reported decreased specificity with more recovery of 50% of BORSA strains .[6,29]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 genotypicmethods.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.[7]

Genotyping methods like PFGE, could one of the best way to discriminate between MSAA,BORSA and MRSA as documented by one of the group of studies. Many BORSA strains belonged to ST25 lineage. [6,30.]Besides this, other promising studies involving various genotyping methods like PFGE, MLST and spa typing haddemonstrated 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,fluroquinolones etc. In the present academic study, most of the cases were treated by ceftriaxone, piperacillin/tazobactum, clindamycin,doxycycline and imipenem. Similar treatment choices are also mentioned by equivalent studies.[7] 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/tazobactum 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 bythe lack of a significant increase in the growth inhibitionzones around cefotaxime–clavulanic acid and ceftazidime,recorded therapeutic success with shifting the treatment from oxacillin to vancomycin.[6,25]Similarly, one deathof 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 out 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.[11]In the present work, 2 beta hemolytic without pigment,1 golden yellow pigmented as well as beta hemlolytic 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 invivo 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 hemolysisetc 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).β-lactamase enzyme is encoded by the blaZ gene, is categorized as a class A β-lactamase (penicillinase) which shows low hydrolytic activity against oxacillin, cephems and carbapenems.

Many clinical microbiology laboratories use only the cefoxitin test for detection of oxacillin resistancein *Staphylococcus spp*. Indeed, the cefoxitin test is a marker of resistance to oxacillin by acquisition of the *mecA gene* butit is unable to detect borderline resistant strains. In our study, cefoxitin,oxacillin and amoxyclav were used to detect such strains. Similar suggestion are quoted by many school of thoughts, especially for *Staphylococcus aureus* and other staphylococci.[4-7] I do recommend to use combination of these discs, in my words "**Triad Panel**" to identify borderline resistance inall staphylococci and likewise inmicrococci, macrococci and other gram-positivecocci and represented as well with better sensitivity and specificity in other studies. [4-7] Inthe present study, all isolated clinical strains were borderline resistant (BHPs) and responded well to beta lactums. It very crucial to identifyit 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, linezolidetc 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 amoxyclav though few studies had mentioned about it. [4] 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 amoxyclav 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.[3-7,30]

REFERENCES

[1] B.O. Olayinka, A.T. Olayinka, A.F. Obajuluwa,J.A.Onaolapo,P.F. Olurinola. Absence of *mecA*gene in methicillin-resistant S*taphylococcus aureus* isolates. Afr. J. Infect. Dis. 2009; 3(2):49 – 56.

[2] Vladimir Gostev , Olga Kalinogorskaya, Ksenia Ivanova ,Ekaterina Kalisnikova,Irina Lazareva ,Polina Starkova et al. In vitro selection of high-level beta-lactam resistance in methicillin-susceptible *Staphylococcus aureus*. Antibiotics.2021; 10, 637.

https://doi.org/10.3390/antibiotics10060637

[3] Ryo Nomura, Hidemasa Nakaminami, Kotaro Takasao, Shuna Muramatsu, Yuji Kato, Takeaki Wajima et al. A class A β-lactamase produced by borderline oxacillin-resistant *Staphylococcus aureus* hydrolyses oxacillin. Journal of Global Antimicrobial Resistance.2020; 22: 244–247.

http://dx.doi.org/10.1016/j.jgar.2020.03.002

[4] Uzal Umar, Umar Ahmed Faruk, Damoroem M. Tanko, Mohammed B. Yerima. Clinical isolates of *Staphylococcus aureus* show variation in *β*-lactamase production and are more susceptible to antibiotics conjugated with *β*-lactamase inhibitors. Open Journal of Medical Microbiology. 2016; 6:143-149.

http://dx.doi.org/10.4236/ojmm.2016.64019.

[5] M. Angeles Argudín,S. Roisin, L. Nienhaus, M. Dodémont, R. de Mendonça, C. Nonhoff. Genetic diversity among *Staphylococcus aureus* isolates showing oxacillin and/or cefoxitin resistance not linked to the presence of *mec*genes. Antimicrobial Agents and Chemotherapy. 2018;62(7): e00091-18.

https://pubmed.ncbi.nlm.nih.gov/29661881/

[6] Maria M. Hryniewicz, Katarzyna Garbacz. Borderline oxacillin-resistant *Staphylococcus aureus* (BORSA) – a more common problem than expected? Journal of Medical Microbiology. 2017; 66:1367–1373.

https://pubmed.ncbi.nlm.nih.gov/28893360/

[7] Senda MezghaniMaalej,aFaouziaMahjoubiRhimi,a Marguerite Fines,b Basma Mnif,a Roland Leclercq, Adnene Hammami. Analysis of borderline oxacillin-resistant

Staphylococcus aureus (BORSA) strains isolated in Tunisia. 2012 ;50 (10): 3345–3348.

https://pubmed.ncbi.nlm.nih.gov/22814459/

[8] Santhosh D V, Shobha K L ,Bairyl,Gowrish R ,D'Souza J. Phenotypic detection and rate of nasal carriage of heterotypic borderline oxacillin resistant *Staphylococcus aureus* in pre-clinical medical students from Malaysia. Journal of Clinical and Diagnostic Research. 2008; 2:985-990.

https://doi.org/10.7860/JCDR/2008/.294

[9] Eric Ransom, Carey-Ann Burnham. Laboratory detection of borderline-oxacillin resistant *Staphylococcus aureus* (BORSA.) LGM News. 2020-2021;7(2):1-6.

https://pathology.wustl.edu/wp-content/uploads/2021/01/LGM-7.2.pdf

[10] Robert Skov, David R. Lonsway, Jesper Larsen, Anders Rhod Larsen, Jurgita Samulionienė, Brandi M. Limbago. Evaluation of methods for detection of β-lactamase production in MSSA. AntimicrobChemother. 2021; 76(6): 1487–1494.

https://pubmed.ncbi.nlm.nih.gov/33615356/

11.Tammy L.Bannerman, Sharon J Peacock. *Staphylococcus,Micrococcus* and other catalase positive cocci. In Patrick, R., Baron E. J., Laundry, M. L., & Pfaller, M. A, editors. Manual of clinical microbiology, 9th ed, vol 1: ASM Press, Washington, DC;2007, p.390-411.

12.Clinical and Laboratory Standards Institute. 2022. Performance standards for antimicrobial susceptibility testing; 17th informational supplement.M100-M02, M07, and M11. vol. 42, Number 2. Clinical and Laboratory Standards Institute, Wayne, PA.

https://clsi.org/media/wi0pmpke/m100ed32.

13.Jana M.Swenson,Jean B Patel,JamesH.Jorgensen.Special phenotypic methods for detecting antibacterial resistance.In Patrick, R., Baron E. J., Laundry, M. L., &Pfaller, M. A,editors. Manual of clinical microbiology, 9th ed, vol 1: ASM Press, Washington, DC;2007, p.1173-1192.

14.James H.Jorgenson,Joen D Turnidge.Susceptibility test methods:dilution and disk diffusion methods. In Patrick, R., Baron E. J., Laundry, M. L., & Pfaller, M. A,editors. Manual of clinical microbiology, 9th ed, vol 1: ASM Press, Washington, DC;2007, p1152-1172.

15.Hussain FM, Boyle-Vavra S, Daum RS. Community-acquired methicillin-resistant Staphylococcus aureus colonization in healthy children attending an outpatient pediatric clinic. Pediatr Infect Dis J. 2001; 20:763–767.

https://pubmed.ncbi.nlm.nih.gov/11734738/

16. Suggs AH, Maranan MC, Boyle-Vavra S, Daum RS. Methicillin resistant and borderline methicillin-resistant asymptomatic *Staphylococcus aureus* colonization in children without identifiable risk factors. Pediatr Infect Dis J. 1999; 18:410–414.

https://pubmed.ncbi.nlm.nih.gov/10353512/

17. Balslev U, Bremmelgaard A, Svejgaard E, Havstreym J, Westh H.

An outbreak of borderline oxacillin-resistant *Staphylococcus aureus*

(BORSA) in a dermatological unit. Microb. Drug Resist.2005;11:78–81.

https://pubmed.ncbi.nlm.nih.gov/15770100/

18.Kernodle DS, Classen DC, Stratton CW, Kaiser AB. Association of borderline oxacillin-susceptible strains of *Staphylococcus aureus* with surgical wound infections. J. Clin. Microbiol.1998;36:219 –222.

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC124838/

19. Khorvash F, Mostafavizadeh K, MobasherizadehS.Frequency of

*mecA*gene and borderline oxacillin-resistant *Staphylococcus aureus* in nosocomial

acquired methicillin resistance *Staphylococcus aureus* infections. Pak. J. Biol. Sci.2008; 11:1282–1285.

https://pubmed.ncbi.nlm.nih.gov/18819540/

20. Kragh Thomsen M, et al.Clonal spread of *Staphylococcus aureus* with reduced susceptibility to oxacillin in a dermatological hospital unit.Acta Derm.Venereol.2006; 86:230 –234.

https://pubmed.ncbi.nlm.nih.gov/16710581/

21. Leahy TR, et al. 2011. Epidemiology of borderline oxacillin-resistant *Staphylococcus aureus* in pediatric cystic fibrosis. Pediatr. Pulmonol.2011;46:489–496.

https://pubmed.ncbi.nlm.nih.gov/21337531/

22. Skinner S, Murray M, Walus T, Karlowsky JA. Failure of cloxacillin in treatment of a patient with borderline oxacillin-resistant *Staphylococcus aureus* endocarditis. J. Clin. Microbiol.2009; 47:859–861.

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2650896/

23. Nelson L, C. S. Cockram, G. Lui, R. Lam, E. Lam, R. Lai, M. Ip.

Community case of methicillin-resistant *Staphylococcus aureus* infection.

Emerg. Infect. Dis.2006; 12:172–174.

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3291382/

24. Clinical and Laboratory Standards Institute. 2007. Performance standards

for antimicrobial susceptibility testing; 17th informational supplement.

M100-S17, vol. 27, no. 1. Clinical and Laboratory Standards Institute,

Wayne, PA.

25. Felten A, Grandry B, Lagrange PH, Casin I. Evaluation of three techniques for detection of low-level methicillin-resistant *Staphylococcus aureus* (MRSA): a disk diffusion method with cefoxitin and moxalactam, the Vitek 2 system, and the MRSA-screen latex agglutination test. J Clin Microbiol. 2002; 40:2766–2771.

https://pubmed.ncbi.nlm.nih.gov/12149327/

26. Swenson JM, Lonsway D, Mcallister S, Thompson A, Jevitt L et al. Detection of mecA-mediated resistance using reference and commercial testing methods in a collection of *Staphylococcus aureus* expressing borderline oxacillin MICs. DiagnMicrobiol Infect Dis 2007; 58:33–39.

https://pubmed.ncbi.nlm.nih.gov/17240109/

27. Krupa P, Bystro_n J, Bania J, Podkowik M, Empel J et al. Genotypes and oxacillin resistance of *Staphylococcus aureus* from chicken and chicken meat in Poland. Poult Sci. 2014; 93:3179–3186.

https://pubmed.ncbi.nlm.nih.gov/25352679/

28. Krupa P, Bystron J, Podkowik M, Empel J, Mroczkowska A.. Population structure and oxacillin resistance of *Staphylococcus aureus* from pigs and pork meat in South-West of Poland. Biomed Res Int. 2015; 141475:1–9.

https://pubmed.ncbi.nlm.nih.gov/26064878/

29. Brennan GI, Herra C, Coleman DC, O'Connell B, Shore AC. Evaluation of commercial chromogenic media for the detection of methicillin-resistant *Staphylococcus aureus*. J Hosp Infect. 2016; 92:287–292.

https://pubmed.ncbi.nlm.nih.gov/26679725/

30.Nadarajah J, Lee MJ, Louie L, Jacob L, Simor AE. Identification of different clonal complexes and diverse amino acid substitutions in penicillin-binding protein 2 (PBP2) associated with borderline oxacillin resistance in Canadian *Staphylococcus aureus* isolates. J Med Microbiol. 2006; 55:1675–1683.

https://pubmed.ncbi.nlm.nih.gov/17108271/

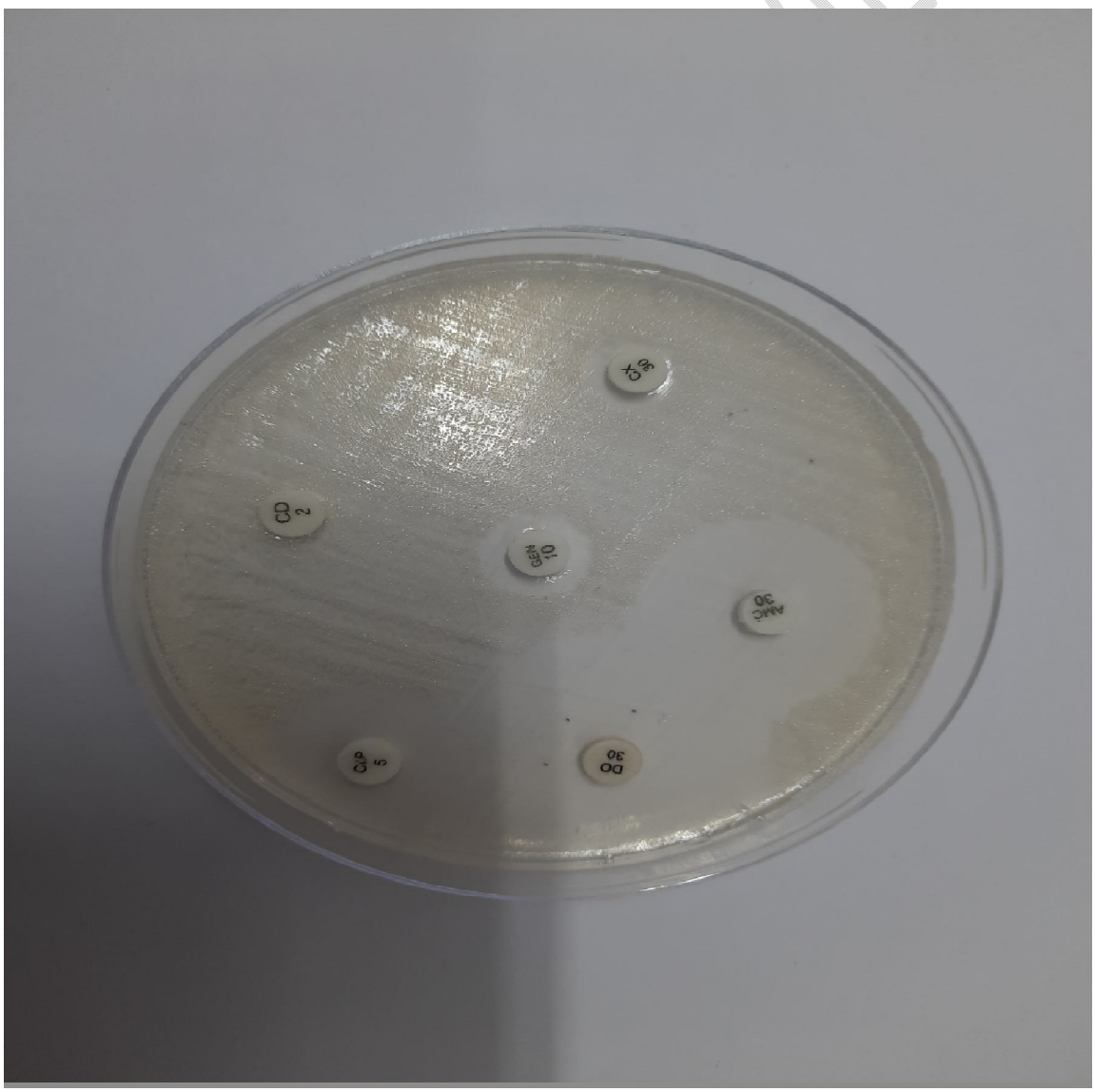

FIGURE 1 SHOWING REDUCED SUSCEPTIBILITY TO CEFOXITIN (CX) AND SUCEPTIBLE ZONE OF AMOXYCLAV (AMC)