**Borderline oxacillin-resistant *Staphylococcus aureus* (BORSA) – a more common problem than expected?**

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**Abstract**

Borderline oxacillin-resistant *Staphylococcus aureus* (BORSA) represents a quite poorly understood and inadequately defined phenotype of methicillin resistance. BORSA strains show low, borderline resistance to penicillinase-resistant penicillins (PRPs), with oxacillin MICs typically equal to 1–8 µg ml\(^{-1}\), and in contrast to methicillin-resistant *S. aureus* (MRSA), do not have an altered penicillin-binding protein, PBP2a, encoded by the *mecA* or *mecC* gene. Their resistance is typically associated with hyperproduction of beta-lactamases or, in some cases, point mutations in PBP genes. BORSA cannot be classified as either truly methicillin-resistant or truly methicillin-susceptible strains. However, they are frequently misidentified, which poses an obvious epidemiological and therapeutic threat. BORSA strains are commonly isolated from humans and animals, and are found both in hospitals and in a community setting. The epidemiology and clinical presentation of BORSA infections seem to be similar to those for MRSA; these infections are usually more severe than those caused by methicillin-sensitive *S. aureus* (MSSA). Treatment of severe infections caused by BORSA may be ineffective, even with larger doses of oxacillin. The available evidence suggests that BORSA represent a frequently neglected problem, and their emergence in new environments implies that they need to be monitored and accurately distinguished from MSSA and MRSA.

**METHICILLIN RESISTANCE IN *STAPHYLOCOCCUS AUREUS***

*Staphylococcus aureus* is one of the most common human pathogens; staphylococcal infections vary in severity, from mild dermatitis to life-threatening sepsis, endocarditis and toxic shock syndrome. Beta-lactam antibiotics still remain the treatment of choice for staphylococcal infections. Penicillin, the first antibiotic from this group, was initially released of this antibiotic in 1942, and shortly thereafter penicillins, with oxacillin MICs typically equal to 1–8 µg ml\(^{-1}\), and in contrast to methicillin-resistant *S. aureus* (MRSA), do not have an altered penicillin-binding protein, PBP2a, encoded by the *mecA* or *mecC* gene. Their resistance is typically associated with hyperproduction of beta-lactamases or, in some cases, point mutations in PBP genes. BORSA cannot be classified as either truly methicillin-resistant or truly methicillin-susceptible strains. However, they are frequently misidentified, which poses an obvious epidemiological and therapeutic threat. BORSA strains are commonly isolated from humans and animals, and are found both in hospitals and in a community setting. The epidemiology and clinical presentation of BORSA infections seem to be similar to those for MRSA; these infections are usually more severe than those caused by methicillin-sensitive *S. aureus* (MSSA). Treatment of severe infections caused by BORSA may be ineffective, even with larger doses of oxacillin. The available evidence suggests that BORSA represent a frequently neglected problem, and their emergence in new environments implies that they need to be monitored and accurately distinguished from MSSA and MRSA.

Another milestone was the development of resistance to methicillin, a penicillin that is not susceptible to the staphylococcal enzymes that hydrolyze the beta-lactam ring. The first case of methicillin resistance in *Staphylococcus* was reported in the United Kingdom as early as 2 years after market authorization for this antibiotic [2, 3].

Together with nafcillin, oxacillin and cloxacillin, methicillin belongs to the penicillinase-resistant penicillins (PRPs), i.e. antibiotics that are not susceptible to degradation with staphylococcal beta-lactamases. Resistance to methicillin is usually associated with the presence of the alternative penicillin-binding proteins, PBP2a or PBP2′, encoded by the chromosomal genes *mecA* or *mecC* (*mec\(_{ALGA251}\)*) [4, 5]. Thw staphylococcal PBPs are transpeptidases that are involved in the transport of peptide precursors for peptidoglycans (D-alanyl-D-alanine termini) from the cytoplasm to the cell membrane [6].

Modified PBP2a has a low affinity to beta-lactams and therefore takes over the function of other PBPs that have been inactivated by these antibiotics. Due to the presence of the new protein, methicillin-resistant *S. aureus* (MRSA) strains can normally synthesize peptidoglycans and cross-link them within the cell wall; as a result, such
strains are no longer susceptible to beta-lactams and show resistance to antibiotics from many other groups [7, 8].

Depending on the proportion of staphylococcal cells with mutant proteins within a given population, resistance to methicillin can be homogeneous or heterogeneous; in other words, the resistant phenotype can be expressed by all bacterial cells or only a fraction thereof. The strains with either a homogeneous or a heterogenous pattern of methicillin resistance show clinical resistance to beta-lactam antibiotics [9, 10].

MRSA are typically isolated in a hospital setting; such hospital-acquired MRSA (HA-MRSA) are believed to spread primarily via human-to-human transmission [11]. However, an increase in the incidence of infections caused by non-nosocomial MRSA, referred to as community-acquired MRSA (CA-MRSA) has been also observed in the last decade, especially in nursing homes, healthcare centres, kindergartens and schools [12, 13]. Importantly, infections caused by these pathogens are usually more severe because CA-MRSA can synthesize Panton–Valentine leukocidin (PVL) [13–15].

Since the isolation of the first methicillin-resistant strain of S. aureus, we have observed a constant increase in the incidence of MRSA infections, and these pathogens remain the most important challenge in terms of drug resistance, from both the clinical and epidemiological perspectives [2, 9, 16].

**DEFINITION AND PROPERTIES OF BORDERLINE OXACILLIN-RESISTANT S. AUREUS (BORSA)**

Apart from the synthesis of modified PBP2a, staphylococcal resistance to methicillin may be also determined by other mechanisms. One example is the staphylococcal strains referred to as borderline oxacillin-resistant S. aureus (BORSA); they show low, borderline resistance to PRPs and unlike MRSA do not carry modified PBP2a encoded by the mecA or mecC gene. BORSA strains cannot be classified as either methicillin-resistant or methicillin-susceptible; their resistance, usually less pronounced than the one determined by PBP2a, results from the involvement of other, sometimes not completely understood mechanisms [6, 17, 18].

Initially, BORSA were defined as the strains with an oxacillin MIC of \( \leq 2 \mu g \text{ ml}^{-1} \) and full susceptibility to PRPs [19]. However, further studies demonstrated that the oxacillin MIC for some BORSA strains may be higher (4–16 \( \mu g \text{ ml}^{-1} \)) and they are not necessarily susceptible to larger doses of PRPs *in vivo* [20].

Borderline oxacillin resistance in S. aureus may be determined by various biochemical mechanisms. The first documented underlying mechanism of borderline resistance was the hyperproduction of beta-lactamase. McDougal and Thornsberry demonstrated that some S. aureus strains synthesize particularly large amounts of this enzyme, which can degrade penicillin (the most hydrolysis-prone antibiotic from this group) rapidly, but inactivates other beta-lactams, including PRPs, slowly [21].

A certain portion of staphylococcal beta-lactamases is released into the surrounding medium or bound to the bacterial surfaces. Most beta-lactamases are encoded by plasmid genes, usually those carried by class II plasmids [22]. Most S. aureus strains synthesize inducible beta-lactamases in the presence of an antibiotic. The rate of synthesis is strain-specific and depends on growth conditions [1]. Usually, hospital staphylococcal strains produce larger amounts of beta-lactamases, releasing 40–60 % of the synthesized enzyme into the growth medium. The synthesis of beta-lactamase and its release to the extracellular environment can be also enhanced by the addition of 5–10 % NaCl [23].

If the resistance of BORSA strains to PRPs results from hyperproduction of beta-lactamase, it can easily be counterbalanced with inhibitors of this enzyme, 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 [21].

However, the addition of a beta-lactamase inhibitor does not necessarily restore the susceptibility of BORSA strains to oxacillin. This problem has *inter alia* been described in S. aureus isolates from cystic fibrosis patients, in which case the addition of clavulanic acid to the ampicillin disc did not alter the size of the growth inhibition zone [24]. In turn, Skinner et al. isolated a BORSA strain whose susceptibility to ceftotaxime and ceftazidime remained unaltered despite the addition of clavulanic acid [20]. Although this mechanism of resistance to PRPs has not yet been established, it is definitely not associated with the hyperproduction of penicillinase. According to the above-mentioned authors, resistance to beta-lactam inhibitors may be associated with the presence of a modified PBP.

These findings suggest that the hyperproduction of conventional penicillinase is not the only cause of the borderline resistance. Indeed, other mechanisms have been implicated as possible determinants of the BORSA phenotype, including the synthesis of new, plasmid-encoded beta-lactamases or the modification of PBP genes resulting from spontaneous amino acid substitutions in the transpeptidase domain [25–27]. These modifications typically involve PBP3 and/or PBP4, and result from the selective pressure of beta-lactam antibiotics. BORSA strains that carry such modifications constitute a particular threat from both the epidemiological and therapeutic perspectives. To be distinguished from beta-lactamase-hyperproducing BORSA, they are referred to as modified S. aureus (MODSA) [28]. Similar to the MRSA strains, MODSA do not become susceptible to PRPs after the addition of a beta-lactamase to oxacillin.

Another feature of BORSA strains that may contribute to their misidentification and resultant therapeutic difficulties,
is a lack of species-specific proteins, such as thermonuclease or coagulase. Recently, Skinner reported infection caused by the strain showing borderline resistance to oxacillin and lacking either thermonuclease or coagulase, i.e. two basic taxonomic characteristics of \textit{S. aureus}. Aside from difficulties in the selection of an appropriate antibiotic therapy, another challenge was identification of the isolate at a species level [20].

Lacking the expression of species-specific proteins has primarily been reported in the case of MRSA; this phenomenon may result from the insertion of transposon (Tn917) or the integration of plasmid genes into the chromosome [29, 30]. According to Duval-Ifrah et al. the loss of the ability to coagulate plasma may result from lysogenic conversion with LS1 and LS2 phages [31]. However, the reasons behind this phenomenon in BORSA strains are not completely understood, and this issue requires further research.

PREVALENCE AND PATHOGENICITY OF BORSA STRAINS

The infrequent identification of the BORSA phenotype seems to be primarily associated with the use of diagnostic methods aimed at the non-selective isolation of all MRSA-like strains, without an insight into the exact mechanism of their resistance. The prevalence of BORSA strains is approximately 5% on average, but may vary from population to population (1.4%–12.5%) [6, 12, 32]. However, some authors have reported substantially higher prevalence rates for BORSA. According to Khovash et al. MRSA strains without the \textit{mecA} gene represented up to 25.5% of all the isolates were initially designated as MRSA [33]. In another study, the prevalence of BORSA was estimated at up to 50% of all clinical isolates of \textit{S. aureus} [24]. Given the limited information available concerning the MRSA detection methodologies employed in previously published studies, some of these strains might in fact display borderline resistance to methicillin [24].

The exact prevalence of BORSA strains is difficult to establish and has not been determined unequivocally thus far. The carriage rates of BORSA strains have only been the subject of a few previously published studies. Nevertheless, these micro-organisms seem to colonize the nares in asymptomatic healthy subjects. In a study of 500 healthy children, staphylococcal strains with the BORSA phenotype were isolated from more than 5% of the subjects [34, 35]. In another study examining staphylococcal infections in animals, some isolates turned out to originate from animal keepers who were asymptomatic carriers of BORSA [36].

BORSA were found in both hospital and community settings. To date, strains with this resistance phenotype have been isolated from skin and soft tissue infections [32, 37], surgical wounds [38], airways [24, 33] and the urinary tract [39]. However, BORSA may be also involved in the etiopathogenesis of some more severe infections, such as endocarditis and sepsis [20]. In a hospital setting, BORSA are most frequently isolated in dermatology units [32, 37, 39].

The symptomatology of BORSA infections seems to be similar to that in the case of MRSA; the infections are usually more severe than those caused by MSSA. In a study conducted by Balslev et al., patients from a dermatology unit who had been infected with BORSA presented with more severe symptoms, more often required rehospitalization and had more bed days than MSSA-infected controls [32].

Owing to similar clinical presentation, infections caused by BORSA may sometimes be misdiagnosed as MRSA (particularly CA-MRSA) infections. Similar to CA-MRSA, BORSA may cause community-acquired soft-tissue infections and necrotic pneumonia, and their isolation may be associated with a history of previous antibiotic therapy [40–42].

Many researchers point to the administration of antibiotics as the main risk factor for developing the BORSA resistance phenotype. In one study, patients with cystic fibrosis who had previously been treated with oral cephalosporin and inhaled tobramycin due to MSSA infections, turned out to be particularly prone to colonization with BORSA. According to the authors of this study, BORSA strains might develop due to selective antibiotic pressure, as a variant of methicillin-resistant strains [24]. Indeed, BORSA isolates from cystic fibrosis patients showed a low degree of strain relatedness and presented with variable PFGE patterns. This implies that they were unlikely to have been transmitted from patient to patient, but rather evolved from strains that had previously acquired methicillin resistance [24].

On the other hand, some evidence suggests that BORSA may spread from patient to patient. Studies involving various genotyping methods (PFGE, MLST and \textit{spa} typing) demonstrated a high degree of strain relatedness in BORSA isolates. In a study conducted by Thomsen et al., up to 93% of the BORSA strains found in a dermatology unit represented the same \textit{spa} type, t230 [37]. According to the authors of this study, the fact that only one clone had been spread selectively across the unit might have been the result of prolonged preferential use of beta-lactams (dicloxacillin) and direct patient-to-patient contacts [37]. In line with these findings, Nadarajah et al. demonstrated that most BORSA isolates showed the same PFGE A pattern, corresponding to the ST25 lineage, which was not related to any of the five clonal complexes found in MRSA [43]. Similarly, more than 80% of the BORSA isolates from equine wound infections represented the same clonal complex, ST-1/2863, which probably emerged as a result of the routine preoperative prophylactic administration of penicillin [36].

PREVALENCE OF BORSA IN ANIMALS

The prevalence of staphylococci with borderline resistance to oxacillin is not limited solely to the human population. BORSA have been also isolated from cattle, horses and...
poultry, as well as other livestock [36, 44–47]. The prevalence and genotype of animal BORSA isolates vary depending on geographic latitude, host species and breeding system. In one study, up to 14% of *S. aureus* isolates from the nasal cavity of pigs kept at two farms showed borderline resistance to oxacillin [46]. The evaluation of staphylococcal infections occurring in horses treated at a Swiss clinic documented even an higher (more than 25%) contribution of BORSA strains [36]. Importantly, most equine infections recorded at this clinic were caused by one predominant clone. Staphylococci with borderline resistance to oxacillin might also contribute to a certain proportion of infections in poultry (chickens and turkeys) [45].

BORSA were also isolated from foods. A number of previous studies demonstrated the presence of this pathogen in ruminant milk, and a few authors have isolated BORSA strains from animal meat. The carriage of BORSA among livestock was shown to be linked with the further isolation of these strains from animal products. The isolation of BORSA from foods was first reported in 2010 [48]. However, the origin of the borderline resistant strains found in foods is still unclear. Presumably they originate from two sources. Isolates from some products that have been processed by humans, such as minced pork and ruminant milk, showed genetic relatedness to strains isolated from food industry workers [24, 49]. However, the carriage of BORSA among livestock may also contribute to the presence of these strains in foods of animal origin [46]. Consequently, both humans and animals may constitute a source of infection and contribute to the contamination of food with BORSA.

Presumably, as in MRSA, one reason for the emergence of BORSA in the animal population and foods is the widespread use of antibiotics. A growing body of evidence is documenting the presence of MRSA strains in livestock and the possibility of their transmission to humans, as livestock-associated MRSA (LA-MRSA) or farm-associated MRSA (FA-MRSA) [50–54]. The selection of these multidrug-resistant staphylococci is undoubtedly promoted by the widespread use of antibiotics as a component of animal fodder. For example, nearly 80% of all antibiotics consumed in the United States are used for animal feeding purposes [55]. According to Casey et al., livestock operations requiring frequent contact with pigs are associated with 38 and 30% greater risk of CA-MRSA and HA-MRSA infection, respectively. To the best of our knowledge, no equally accurate data regarding the risk of infection with BORSA strains have been published thus far.

**DETECTION OF BORSA STRAINS**

The detection of BORSA in clinical material is a prerequisite for effective antibiotic therapy. Unfortunately, BORSA detection methods that can be used in routine diagnostics remain to be developed. Due to the lack of such methods, some BORSA strains may be misdiagnosed as MRSA or MSSA isolates. The risk of the misdiagnosis is undoubtedly associated with the use of routine protocols to detect methicillin resistance. In line with the recommendations of the European Antimicrobial Resistance Surveillance System (EARSS), since 2005 cloxacinil discs (1 µg) have been replaced by cefoxitin discs (30 µg). An *S. aureus* isolate is considered to be resistant if the inhibition zone around the cefoxitin disc is no greater than 21 mm. According to the Clinical and Laboratory Standards Institute (CLSI) guidelines from 2009, the strain is classified as MRSA whenever its oxacillin MIC amounts to ≥8 µg ml⁻¹ [56].

Susceptibility to cefoxitin is also 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 the latter involves a different mechanism than the presence of PBP2a (*mecA/C* gene). BORSA strains are identified as methicillin-sensitive and methicillin-resistant based on the size of the inhibition zone around cefoxitin (30 µg) and oxacillin (1 µg) disc, respectively [39]. If only susceptibility to cefoxitin is tested, a BORSA strain may be misidentified as an MSSA isolate. Therefore, it is recommended that both cefoxitin and oxacillin susceptibility be tested for the detection of BORSA in a laboratory setting [39, 57, 58].

In most *S. aureus* strains, borderline resistance to oxacillin is determined by the hyperproduction of beta-lactamases [24, 45, 46]. The involvement of this mechanism is typically confirmed by a cefinase test with a nitrocefin disc. Alternatively, the bactericidal/bacteriostatic activity of a beta-lactamase inhibitor, e.g. clavulanic acid, in combination with a PRP can be determined. BORSA strains become fully susceptible to oxacillin in the presence of a beta-lactamase inhibitor [59] and, consequently, both their MIC and the size of the inhibition zone have values that are typical for a sensitive strain. However, both of these parameters remain unchanged in the case of either methicillin-sensitive or methicillin-resistant strains [20].

Sometimes distinguishing between BORSA and MRSA strains may be challenging due to overlapping oxacillin MICs [12]. In line with the CLSI guidelines, a strain can be classified as MRSA if its oxacillin MIC is equal to 8 µg ml⁻¹ or higher [56]. However, some MRSA may have oxacillin MICs below 8 µg ml⁻¹, and MICs for some BORSA isolates may exceed this cut-off value [20, 24]. Published evidence suggests that in such cases, qualification of a strain as BORSA/MRSA should be based on determination of *PBP2a* with latex agglutination and/or detection of *mecA and meCC* genes by means of PCR. In the case of BORSA strains, both these tests produce negative results [60, 61].

According to the literature, unlike CA-MRSA isolates, BORSA strains lack the PVL locus responsible for the expression of the Panton–Valentine toxin [26]. However, the data available for this matter are still sparse.

Selective chromogenic media are increasingly being used for the one-step isolation and identification of MRSA.
The growing popularity of such media is associated with the need for the rapid detection and identification of CA-MRSA and HA-MRSA. Early detection is crucial for adequate control of their spread, and improves the effectiveness of treatment while shortening its duration. However, the growing popularity of chromogenic media has stimulated questions about their sensitivity and specificity in distinguishing between various phenotypes of methicillin resistance in *S. aureus*, including BORSA.

A recent study included four chromogenic media for the detection of MRSA – MRSA Select II (BioRad, USA), Colorex MRSA (E&O Laboratories, UK), ChromID MRSA (bioMerieux, France) and MRSA Brilliance 2 (Oxoid, UK) – and confirmed their high (nearly 100%) sensitivity but markedly lower specificity [62]. The reduced specificity of the chromogenic agars was demonstrated by the high recovery of BORSA, with almost 50% false positive results on all four media. The authors of this study linked this result to the high values of oxacillin MIC (4–8 µg ml⁻¹) for the analysed BORSA strains. Misdiagnosis of BORSA as MRSA undoubtedly has a negative impact on the control of their spread in hospitals and in a community setting. This implies that strains showing borderline resistance to PRPs should be identified with other, more accurate methods than the chromogenic media.

Another unquestioned obstacle to the accurate identification of BORSA isolates is the previously mentioned lack of species-specific factors, such as coagulase and thermonuclease [20].

**PROBLEMS WITH TREATMENT OF BORSA INFECTIONS**

The detection of *S. aureus* strains with borderline resistance to oxacillin in a clinical material may hinder the selection of an appropriate antibiotic therapy [12, 20]. Initially, infections caused by BORSA with oxacillin MICs ≤2 µg ml⁻¹ were treated with PRPs. BORSA strains were often not considered to be resistant to beta-lactams, despite synthesizing large amounts of beta-lactamas that slowly hydrolyzed these antibiotics *in vitro*. However, the process of hydrolysis was considered to be too slow to have any meaningful clinical implications, such as treatment failure [18, 27].

However, this concept evolved with time. Currently, *mecA*-negative *S. aureus* strains with higher oxacillin MICs (≥4 µg ml⁻¹) are considered to be a therapeutic challenge, and oxacillin and other PRPs, even at higher doses, are known to be inefficient in the treatment of severe BORSA infections. This was unequivocally considered to be the case by Skinner et al. in 2009. The oxacillin MIC for the strain isolated from a patient with endocarditis was 12 µg ml⁻¹ (E-test) and the results of testing for *mecA* and BPP2a were negative. Furthermore, the nitrocefin test showed that the isolate did not produce beta-lactamases, which was further confirmed by the lack of a significant increase in the growth inhibition zones around cefotaxime-clavulanic acid and cefazidime-clavulanic acid discs. While the exact mechanism of oxacillin resistance in this case has not been identified, it undoubtedly did not involve the hyperproduction of penicillinase. After identification of the aetiological factor as BORSA, the patient was switched from oxacillin to vancomycin, which eventually resulted in therapeutic success [20].

However, this does not mean that BORSA infections cannot be treated with beta-lactams. The treatment of choice in such cases still comprises large doses of penicillinase-resistant penicillins (e.g. cloxacinil) or beta-lactams +beta-lactamase inhibitors (e.g. ampicillin/sulbactam), providing earlier determination of their MICs and adequate assessment of symptom severity [12, 63, 64]. Beta-lactams should only be implemented after a substantial decrease in MIC (by at least twofold) or a >5 mm increase in growth inhibition zone around the disc with beta-lactamase inhibitor have been confirmed [20].

A key issue that needs to be determined prior to the implementation of an anti-staphylococcal treatment is distinguishing between BORSA and MRSA. Without differentiating between these two phenotypes of antibiotic resistance, each infection should be treated as MRSA, i.e. with other antibiotics than beta-lactams, e.g. vancomycin. As is widely known, such an approach is not beneficial in patients with severe staphylococcal infections, such as endocarditis or sepsis, where vancomycin has been shown to be inferior to beta-lactams. Whenever the absence of the *mecA* gene is confirmed with an appropriate test, the patient should be diagnosed for BORSA infection; in such cases, beta-lactams may be considered to be a therapeutic option after the oxacillin MIC, synthesis of beta-lactamases or lack thereof and clinical presentation have been taken into account [12, 24].

Isolates with the BORSA phenotype may be multidrug-resistant, i.e. show resistance to at least three antibiotics from various groups. According to the literature, BORSA may show resistance to aminoglycosides, including gentamicin/kanamycin, which is determined by the *(acc(6')-Ie-phi(2')-la)* gene, streptomycin *(str* gene) and trimethoprim *(dfr (A) gene)* [36]. Furthermore, BORSA may show (usually inducible) resistance to macrolides and lincosamides [12, 45, 46].

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