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RESEARCH ARTICLE

Antibiogram of Borderline Oxacillin-Resistant *Staphylococcus aureus* (BORSA) and Modified *Staphylococcus aureus* (MODSA) Isolates From Human and Animals in Assam, India

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Abstract

Objectives: Detection of borderline oxacillin-resistant *Staphylococcus aureus* (BORSA) and modified *Staphylococcus aureus* (MODSA) strains with clinical significance are rare events. They have clinical, community, and livestock-associated significance. We aimed to screen these strains and evaluate the antibiogram through an array of antibiotics in Assam, a northeastern state of India. **Method:** *S. aureus* was confirmed biochemically and at the molecular level. Oxacillin and cefoxitin resistance were screened through the disc diffusion test. Hyperproduction of β-lactamase was screened using ticarcillin-clavulanic acid. PBP2a and PBP expressions were screened through *mecA*, *mecC*, and *blaZ1*, *blaZ2* genes. MIC, MAR, MDR, XDR, and PDR were calculated. **Findings:** A total of 141 *S. aureus* isolates were screened. Altogether, 13 (9.22%) *S. aureus* isolates were *mecA* and/or *mecC* negative with oxacillin and/or cefoxitin resistance. Among the only oxacillin-resistant isolates, 33.33% (2/6) of the community-associated and 66.67% (2/3) of the livestock-associated isolates showed multiple drug resistance (MDR) with MAR index > 0.2. All the community-associated isolates showed resistance to oxacillin, while 83.33% showed resistance to ciprofloxacin & ticarcillin-clavulanic acid and 66.67% to fusidic acid. The livestock-associated isolates were 100% resistant to oxacillin, methicillin, and ticarcillin-clavulanic acid. All the oxacillin with cefoxitin-resistant isolates were MDR. Only one XDR was detected. One community-associated isolate was found to be BORSA, whereas the rest were MODSA. Altogether 61.54% had MAR index >0.2. Fusidic acid (0.064-0.125 μg/ml) had the

lowest range of MIC, whereas trimethoprim, rifampicin, penicillin, and cefoxitin had higher ranges of MIC. **Novelty:** To the best of the authors' knowledge, BORSA and MODSA from community and livestock-associated samples are not reported from this region. We assessed the antibiogram against 28 antibiotics (19 classes) and MIC against 12 antibiotics (11 classes). Reporting of MODSA strain from human community samples and livestock samples has great clinical importance from both human and veterinary perspectives.

Keywords: MODSA; MDR; XDR; MAR; MIC; BORSA

1 Introduction

Antimicrobial resistance caused almost 4.95 million deaths in 2019 as estimated by World Health Organisation[(1)]. *Staphylococcus aureus* is one of the six pathogens (*Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa*, and *Enterobacter* species) tagged as pathogens of critical and high importance by WHO, which mostly cause nosocomial infections worldwide. This group of pathogens is highly divergent and antibiotic-resistant, getting the eponymous name "ESKAPE"[(2)]. MRSA infection has been gradually recognized as a universal pandemic worldwide. Though methicillin is no longer commercially available, the acronym MRSA is retained due to its historic importance. MRSA was on the "high" alert group earlier and remains in the "high" alert group as of today in the "Bacterial Priority Pathogen List, 2024" released by WHO[(1)].

A newer version of *S. aureus* was reported worldwide, though in less volume, which was genotypically *mecA* negative but phenotypically oxacillin and/or cefoxitin resistant. It was attributed mainly to the hyperproduction of normal staphylococcal β-lactamase or the modification of the normal PBP with reduced affinity to β-lactams[(3)]. Strains with hyperproduction of normal staphylococcal β-lactamase are termed as borderline oxacillin-resistant *S. aureus* (BORSA) whose MIC value normally falls between 0.5–4.0μg/ml[(4)]. This type of strain can be screened by combining β lactam antibiotics with β-lactamase inhibitors such as clavulanic acid or salbactam[(4)]. Ender *et al.* showed that a point mutation in the *mecA* promoter leads to the repression of the expression of *mecA* and subsequently the fitness of the BORSA strain[(5)]. The other strains with the modification of normal PBP can't be screened with the β lactamase inhibitor and are called modified *S. aureus* (MODSA). They need a proper phenotypic characterisation for screening. Due to this type of modification, these phenotypic oxacillin- and/or cefoxitin-resistant strains have resistance to various other groups of antibiotics.

We screened 611 samples from human and animals for isolation and identification of BORSA and MODSA phenotypically and genotypically. Hyperproduction of β-lactamase was counter-checked with ticarcillin-clavulanic acid (TCC) disc and by detecting *blaZ* gene.

2 Methodology

2.1 Collection of samples

In the present study, skin swabs were collected from anterior nares and surgical wounds of inpatients admitted to the Surgery and Gynaecology department of selected tertiary care hospitals in Assam, India, to isolate hospital-associated *S. aureus*. Wound swabs from domesticated animals and animal patients presented for treatment at the veterinary clinical complex of the College of Veterinary Science, AAU, Khanapara, Guwahati; meat samples from poultry slaughterhouses, and bovine milk from farm animals were collected for isolation of livestock-associated *S. aureus*. Samples from

anterior nares were collected from apparently healthy individuals in communities to isolate community-associated *S. aureus*. Except for meat and milk, all other samples were collected with sterile cotton swabs (HiMedia, PW009) in aseptic conditions. Individual milk and meat samples were collected in sterile sample containers (HiMedia, PW126) in an aseptic condition. All swabs were immediately placed in Cary Blair transport medium (SRL, CM012) and processed within 48 hrs of collection. All meat and milk samples were processed immediately after collection.

2.2 Biochemical Confirmation

All collected samples were directly inoculated in Peptone Water Broth (HiMedia, M614) at 35°C overnight, followed by streaking on Mannitol Salt Agar (HiMedia, MH118) supplemented with 2 mg/L cefoxitin. Suspected golden yellow colonies were further confirmed on Baird Parker Agar (HiMedia, M043) supplemented with egg yolk solution (HiMedia, FD045) and Potassium tellurite (HiMedia, FD047), as per manufacturer instructions. Positive *S. aureus* colonies appeared black with a clear halo zone around them due to the lecithinase production. *S. aureus* isolates were confirmed with the tube coagulase test with coagulase plasma (HiMedia, FD248). Tubes with positive *S. aureus* appeared coagulated after incubation.

Genomic DNA was extracted from all *S. aureus* isolates using Presto^{TM} Mini gDNA bacteria kit (Geneid, GBB300). Extracted DNA was quantified in a spectrophotometer (Picodrop, Pico100) and preserved immediately at -20°C for further use.

2.3 Molecular confirmation of *S aureus* isolates

All *S. aureus* isolates were confirmed through the detection of *aroA* and *nuc* genes.

A Primer for *aroA* was designed, and PCR conditions were standardised in-house as mentioned earlier[6]. Detection of *nuc* gene was done using reported[6] primers (10 μM each) and conditions with slight modification of the annealing temperature for the *nuc* gene. The 2X DreamTaq PCR master mix (Thermo Scientific, K1071) was used for the PCR amplification.

Five randomly selected *S. aureus* isolates were subjected to *16sRNA* gene amplification using reported primers[6] and conditions with modification of increasing the annealing temperature from 37°C to 47°C. Amplified products were sent for Sanger sequencing. Nucleotide BLAST (Blastn) was performed with obtained sequences. Primer details are provided in Table 1.

Table 1. Primer pairs used for amplification of different genes

Sl. No.	Gene name	Primer details (5′-3′)	Amplicon size (bp)	Annealing temp. (° C)	References
1	*aroA*	AAGGGCGAAATAGAAGTGCCG ATTGGTTGAAGCATTGGTGT	875	52	[6]
2	*mecC*	GCTCCTAATGCTAATGCA TAAGCAATAATGACTACC	304	50	[7]
3	*mecA*	AGAAGATGGTATGTGGAAGTTAG ATGTATGTGCGATTGTATTGC	584	57	[8]
4	*nuc*	AAGCGATTGATGGTGATACGGTT CAAGCCTTGACGAACTAAAGC	278	50	[6]
5	*16s RNA*	AGAGTTTGATCCTGGCTCAG GGTTACCTTGTTACGACTT	1513	47	[6]
6	*blaZ1*	TAAGAGATTTGCCTATGCTT TTAAAGTCTTACCGAAAGCAG	377	52	[9]
7	*blaZ2*	GTTGCGAACTCTTGAATAGG GGAGAATAAGCAACTATATCATC	674	50	[9]

2.4 Antimicrobial susceptibility test

All *S. aureus* isolates were screened for the presence of *mecA* and *mecC* genes using reported PCR primers and conditions[7,8] for identification of the methicillin-resistant *S. aureus* isolates. All *mecA* and/or *mecC* negative isolates were first screened against oxacillin and cefoxitin by Kirby-Bauer disc diffusion method for phenotypic characterisation.

Bacterial turbidity of 0.5 McFarland constant was achieved with log-phased growth of *S. aureus* isolates. Isolates were smeared on a sterile Mueller Hinton Agar plate using sterile swabs (HiMedia, PW003). Antibiotic discs were placed aseptically and incubated aerobically at 35°C for 18 hours, followed by another 6 hours at the same temperature to screen heteroresistant *S. aureus*. The zone of inhibition was measured for cefoxitin and oxacillin discs after 18 and 24 hours of incubation, respectively.

All *mecA* and/or *mecC* negative but oxacillin and/or cefoxitin non-susceptible isolates were further subjected to 18 antibiotic classes consisting of 26 different antibiotics for qualitative analysis through disc diffusion method. Details of antibiotics used are provided in Table 2. The zone of inhibition was measured and interpretation was done according to CLSI guidelines[10].

All *mecA* and/or *mecC* negative but oxacillin and/or cefoxitin non-susceptible isolates were subjected to 12 antibiotics from 11 different classes for quantitative analysis through E-test using gradient antibiotic strips for the calculation of minimum inhibitory concentration (MIC). Details of antibiotic strips used are provided in Table 3. MIC value was measured, and interpretation was done according to CLSI guidelines[10].

Multi-drug Resistance (MDR), Extensive drug resistance (XDR) and Pan drug resistance (PDR) were determined and reported accordingly[11].

Table 2. Antibiotics used for Kirby Bauer disc diffusion test

Sl no	Antibiotic Class	Antibiotic name	Concentration (μg)
1	Penicillinase stable Penicillin	Methicillin	5
		Oxacillin	1
2	Steroidal	Fusidic acid	10
3	β lactam + β lactamase Inhibitor	Ticarcillin-clavulanic acid	75/10
4	Cephalosporins	Cefaclor (2nd gen)	30
		Cefdinir (3rd Gen)	5
		Cefotaxim (3rd Gen)	30
		Cefepime (4th Gen)	30
5	Glycipeptides	Teicoplanin	30
6	Aminoglycosides	Gentamicin	10
		Kanamycin	30
		Tobramycin	10
7	Macrolides	Erythromycin	15
8	Tetracyclines	Doxycycline hydrochloride	30
		Tetracycline	30
9	Fluoroquinolones	Ciprofloxacin	5
10	Nitrofurans	Nitrofurantoin	100
11	Lincosamides	Clindamycin	2
12	Phenicols	Chloramphenicol	30
13	Pseudomonic acid	Mupirocin	200
14	Ansamycin	Rifampicin	5
15	Oxazolidinones	Linezolid	30
16	Cefamycin	Cefoxitin	30
17	Penems	Imipenem	10
		Meropenem	10
18	Folate pathway inhibitor	Trimethoprim	5
		Co-Trimoxazole	1.25/23.75
19	Streptogramins	Pristinamycin	15

Table 3. Antibiotics used for E test

Sl no	Antibiotic Class	Antibiotics	Range (μg/ml)
1	Steroidal	Fusidic Acid	0.016-256
2	Ansamycin	Rifampicin	0.002-32

Continued on next page

Table 3 continued

3	Tetracyclines	Tetracycline	0.016-256
4	Folate Pathway Inhibitor	Trimethoprim	0.002-32
5	Glycopeptide	Teicoplanin	0.016-256
		Vancomycin	0.016-256
6	Penicillinase stable Penicillin	Oxacillin	0.016-256
7	Oxazolidinones	Linezolid	0.016-256
8	Macrolides	Erythromycin	0.016-256
9	Penicillinase labile Penicillin	Penicillin	0.002-32
10	Lincosamides	Clindamycin	0.016-256
11	Cefamycin	Cefoxitin	0.016-256

2.5 Antibiotic resistance gene characterization

All *mecA* and/or *mecC* negative but oxacillin and/or cefoxitin non-susceptible isolates were further screened for β-lactamase producing genes *blaZ1* & *blaZ2* using reported primers and conditions[9].

2.6 Calculation of MAR index

The Multiple Antibiotic Resistance (MAR) index was calculated as described[12]. A MAR index >0.2 signifies a high risk of contamination and resistance where several antibiotics are used for treatment or growth promotion. A MAR index < 0.2 indicates the use of less antibiotic resistance. MAR index = 1 indicates that the isolate is resistant to all antibiotics screened.

3 Results and Discussion

By screening a total of 611 samples from hospitals, communities, and livestock, 141 isolates of *S. aureus* were obtained. All these isolates were found harboring *aroA* and *nuc* genes (Figure 1). Sequence similarity search by Blastn with *16s RNA* sequences of the isolates clearly identified the coagulase-positive isolates as *Staphylococcus aureus* with > 99% similarity with corresponding sequences of *S. aureus* in the NCBI database. Isolates bearing no *mecA* gene were accepted for further analysis (Figure 1). A total of eight and five *mecA* and/or *mecC* negative *S. aureus* isolates from the community and livestock, respectively, were found to be oxacillin and/or cefoxitin resistant. Details are provided in Table 4.

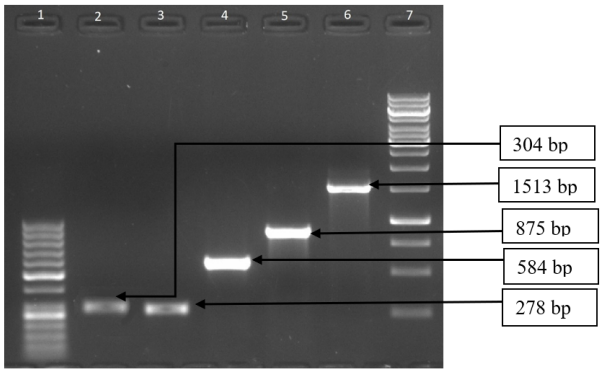

Fig 1. Amplification of different genes. Lane 1- Gene ruler 50bp ladder; Lane 2- *mecC* amplified product; Lane 3- *nuc* amplified product; Lane 4- *mecA* amplified product; Lane 5- *aroA* amplified product; Lane 6- 16s rRNA amplified product; Lane 7- Gene ruler 1kb ladder

Table 4. Isolation summery

Type of isolates	Samples tested	Positivefor *S. aureus*	*mecA*-ve and/or *mecC* -ve samples	Resistant to oxacillin and/or cefoxitin*	Resistant to oxacillin and/or cefoxitin*#
Hospital Associated	154	21 (13.63)	1	0 (0.00)	0 (0.00)
Community Associated	253	57 (22.52)	25	8 (14.03)	8 (32.00)
Livestock Associated	204	63 (30.88)	11	5 (7.93)	5 (45.45)

* Percentage against total *S. aureus* isolate. *# Percentage against total *mecA* –ve and/or *mecC* -ve *S. aureus* isolate. (Figures in parenthesis denote percentages)

All these isolates could be subdivided into two groups, viz., oxacillin resistance and oxacillin and cefoxitin resistance. In the first group, 83.33% of the community-associated isolates were resistant to ticarcillin + clavulanic acid and ciprofloxacin, followed by 66.66% to fusidic acid, 33.33% to pristinamycin, trimethoprim, erythromycin, kanamycin and 16.16% to tobramycin and gentamicin. On the other hand, all (100%) of the livestock-associated isolates showed resistance to ticarcillin + clavulanic acid and methicillin followed by 66.66% to ciprofloxacin, pristinamycin, trimethoprim, erythromycin, kanamycin and 33.33% to fusidic acid. In the second group, all (100%) community-associated isolates showed resistance to ticarcillin+clavulanicacid, ciprofloxacin, fusidic acid, rifampicin, and methicillin, followed by 50% to pristinamycin, trimethoprim, erythromycin, kanamycin, co-trimoxazole, imipenem, linezolid, clindamycin, mupirocin, cefepime, cefotaxime, cefdinir, & cefaclor. On the other hand, all (100%) livestock-associated isolates exhibited resistance to ticarcillin + clavulanic acid, ciprofloxacin, trimethoprim, kanamycin, and methicillin while 50% showed resistance to fusidic acid, pristinamycin, erythromycin, co-trimoxazole, tobramycin, gentamicin, & cefdinir.

All isolates were found to be MDR, with one community-associated isolate being XDR. No isolate was found to be PDR. In aggregate, 38.46% of isolates had a MAR index < 0.2, with 0.071 being the lowest, and the rest 61.54% had a MAR index >0.2, with 0.75 being the highest. The mean of the MAR index in livestock-associated sample was 0.293, while community-associated sample was 0.025 when studying against 28 antibiotics. Details of the MAR indices are shown in Table 5.

Table 5. MAR index data

Sl no	No of Antibiotics resistant (a)	No of Sample	No of Antibiotics screened (b)	MAR index(a/b)
1	2	1	28	0.071429
2	3	1	28	0.107143
3	4	3	28	0.142857
4	7	2	28	0.25
5	8	1	28	0.285714
6	9	2	28	0.321429
7	10	1	28	0.357143
8	11	1	28	0.392857
9	21	1	28	0.75

The *S. aureus* had a MIC range of 0.5-2 μg/ml and 0.125-0.25 μg/ml against oxacillin in respect of community and livestock-associated isolates, respectively. Cefoxitin had a MIC range of 2.0 -64.0 μg/ml and 2.0-4.0 μg/ml respectively against community and livestock-associated isolates. Fusidic acid had the lowest range of MIC with 0.064-0.125 μg/ml and 0.125 μg/ml against community and livestock-associated isolates, respectively. It was observed that community associated isolates bore a higher upper value of MIC than livestock-associated isolates for all antibiotics tested except vancomycin. Vancomycin showed almost identical MIC within the range of 0.5-2.0 μg/ml for all isolates. Details are provided in Table 6. All isolates were found harbouring *blaZ1* and *blaZ2* genes (Figure 2).

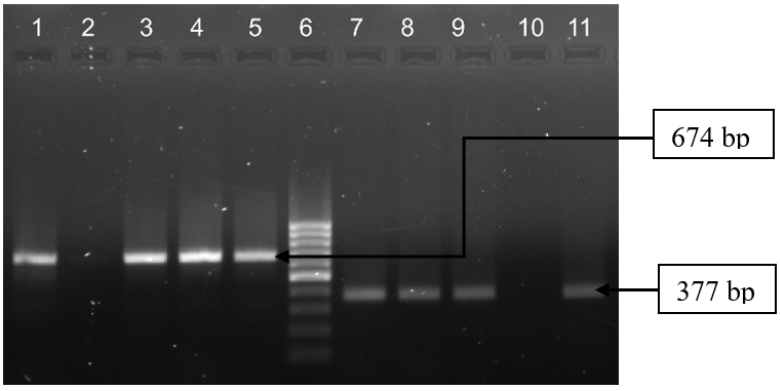

Fig 2. Amplification of *blaZ* gene. Lane 1: *blaZ2* positive control; Lane 2: *blaZ2* negative control; Lane 3-5: *blaZ2* amplified products; Lane 6: NextGen 100bp DNA ladder; Lane 7-9: *blaZ1* amplified products; Lane 10: *blaZ1* negative control; Lane 11: *blaZ1* positive control

Table 6. Obtained range of MIC against antibiotics

Antibiotics	Range of MIC (μg/ml)	
	CA-MRSA(8)	LA-MRSA(5)
Fusidic Acid	0.064 - 0.125	0.125
Rifampicin	0.008 - 4.0	0.004 - 0.008
Tetracycline	0.5 - 1.0	0.25
Trimethoprim	1.0 - 32.0	32.0
Teicoplanin	1.0 - 2.0	2.0
Vancomycin	0.5 - 1.0	2.0
Oxacillin	0.5 - 2.0	0.125 - 0.25
Linezolid	2.0 - 4.0	2.0 - 4.0
Erythromycin	0.5 - 32	0.25 - 16
Penicillin	4.0 - 32.0	0.5 - 1.0
Clindamycin	0.032 - 8.0	0.25
Cefoxitin	2.0 - 64.0	2.0 - 4.0

*Figure in parenthesis denotes number of isolates

Methicillin was the first β-lactamase resistant semi-synthetic penicillin marketed during 1959. Within 2 years of its clinical use, resistance was reported against methicillin[13] from the *Staphylococcus* Reference Laboratory for Phase Typing, Colindale, London, England. Resistance to penicillin was mostly attributed to the production of β-lactamase enzyme by the resistant clones whereas resistance to "β-lactamase-resistant penicillin" was attributed to three mechanisms. The first and the most common mechanism is the production of a low-affinity penicillin-binding protein PBP2a by *mecA* gene or its homolog, *mecC* gene. This group of *S. aureus* is oxacillin and cefoxitin-resistant and predominantly MDR and XDR. We have excluded them from this study, and only *mecA* and/or *mecC* negative isolates were included. The other two mechanisms are accepted as rare events. Secondly, *mecA* or *mecC* negative strains become oxacillin and cefoxitin resistant due to the hyperproduction of β-lactamase enzyme[3]. They also become MDR and XDR. These strains are commonly called BORSA strains. We detected a total of 13 isolates that are *mecA* and/or *mecC* negative. These BORSA strains can be identified by using a β-lactamase enzyme inhibitor combined with β-lactam antibiotics[4]. We used ticarcillin + clavulanic acid disc (TCC). Clavulanic acid deactivates β-lactamase and hence β-lactam can act on that. Most BORSA strains are that's why oxacillin and/or cefoxitin resistant but susceptible to the combination drug. We have screened only a single isolate as BORSA, which was an MDR strain and it had a low MAR index of 0.142. Gareth *et al.* reported 3 patients with BORSA from the orthopaedic ward in Birmingham. Earlier, they reported BORSA strain with ST97 which was *mecA* and *mecC* negative with MIC value of 2-6 μg/ml[14]. In an earlier study from a Netherlands hospital, 0.1% of all *S. aureus* were found to be BORSA with dermatological conditions. Whole genome sequencing showed that the BORSA strain carried multiple resistance and virulence genes with potentially increased pathogenic conditions[15]. Sawhney *et al.* reported 33 BORSA strains from NICU in the USA. It was higher than the expected worldwide report. They noticed 6 particular features which were substitutions and truncations in *GdpP*, *PBP2* & *PBP4* genes and hyperproduction of β-lactamase enzyme making it clearly distinguishable from MRSA[16]. Samsudin *et al.* detected 5 cases of BORSA from bloodstream infection which were later successfully treated with anti-MRSA antibiotics[17]. The third type of resistance is due to the production of the normal PBP but with a reduced affinity towards β-lactam antibiotics. This type of strain is resistant to oxacillin, cefoxitin, and β-lactamase enzyme inhibitors combined with β-lactam antibiotics. We have screened 12 such strains which are oxacillin, and/or cefoxitin and TCC resistant and all harbouring *blaZ1* and *blaZ2* genes which produce normal β lactamase. They are called modified *S. aureus* (MODSA). This finding is in concurrence with that of Gitman *et al.*[18] and Ikpeama *et al*[19]. Non-synonymous substitution mutations in the *pbp* gene of the pbp4 promoter region and/or unique truncating mutations in the gene encoding the cyclic diadenosine monophosphate phosphodiesterase enzyme 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 *mecA* or *mecC* type genes[18,20]. Out of all these isolates, eight community-associated human isolates showed a cefoxitin MIC range of 0.5-2.0 μg/ml. Ideally, a BORSA strain has an oxacillin MIC range of 0.5-4 μg/ml[4]. In this study, we found only one BORSA strain with oxacillin MIC of 1.0 μg/ml and TCC susceptible. The rest of the seven strains were TCC resistant, hence identified as MODSA. It was also found that the MIC range was larger for all antibiotics for community-associated isolates compared to livestock-associated ones with a higher upper value.

All MODSA isolates were found to be MDR with one XDR strain. These isolates have significant clinical importance, being MDR and XDR. Deyno *et al*. screened *S. aureus* from ENT samples of humans and found 100% MDR with MAR index range of 0.22-0.66 using 9 antibiotics from 9 classes[21]. Bai *et al*. found MAR value 0.75-0.86 from chicken farms and < 0.2 from dairy farm, all with MDR against 8 antibiotics[22]. Kasem *et al.*[23] and Urmi *et al.*[24] reported mean MAR values of 0.529 and 0.425 against 14 and 19 antibiotics respectively, while screening *S. aureus* from food items. In a farm-to-fork study approach, Amoako *et al.*[25] reported 38.17% MDR *S. aureus* with a mean MAR value 0.23 while screening against 19 antibiotics. MAR indexing is used universally as a tool or marker to demonstrate antibiotic resistance and spread in the case of *S. aureus* and *E. coli*. In our study, in aggregate, we got 61.54% of strains with MAR index > 0.2. It implies a higher antibiotic resistance in the isolates. The mean of the MAR index in livestock associated (0.293) was found higher than the mean of community-associated samples (0.025). As we have screened against a higher number of antibiotics (28 number from 19 classes), our result gives a better perspective of antibiotic resistance in the BORSA and MODSA strains.

4 Conclusion

Some researchers include MODSA and BORSA as a subgroup of MRSA, whereas others accept it as an entirely new group. This study shows that sole dependence on phenotypic characterization without screening at the molecular level may misjudge them as MRSA. To the best of our knowledge, this is the first study reporting MODSA strains among community and livestock-associated isolates from this region. This study highlights the antibiotic resistance patterns of MODSA strain isolated from human and animal origin. This study also gives insight into the spreading of resistance through MAR indexing. Due to the clinical importance and the typical antibiotic resistance patterns, BORSA and MODSA strains should be studied along with MRSA, phenotypically and genotypically. Further molecular characterization including SCC*mec* typing, and MLST, are required for a better understanding of the epidemiological significance of these strains. These strains can all be further analyzed through Whole genome Sequencing (WGS) and core genome MLST (cgMLST) to understand the virulence and pathogenic characteristics.

Ethical Approval

This study is approved by Institutional Ethics Committee, Gauhati Medical College & Hospital, Guwahati-781032 bearing approval no "MC/190/2007/Pt-11/MAR-2020/19" dated 04/06/2020 and Institutional Ethics Committee, Gauhati University, Guwahati- 781014 bearing approval no "GUIEC/2021/034" dated 29/09/2021.

Informed Consent

There are no experiments done on humans and animals. Consents were obtained for sampling only and it was approved by the concerned ethical committee.

Contributory Statement

- **Conceptualization of study problem**: Naba Kumar Deka, Pratap Jyoti Handique, Probodh Borah
- **Experimental Design:** Naba Kumar Deka, Probodh Borah
- **Data generation:** Naba Kumar Deka, Mridusmita Choudhury, Dipika Malakar
- **Data analysis:** Naba Kumar Deka, Mridusmita Choudhury, Dipika Malakar
- **Research Guidance:** Pratap Jyoti Handique, Probodh Borah
- **Original Drafting:** Naba Kumar Deka, Dipika Malakar
- **Editing & Reviewing:** Pratap Jyoti Handique, Probodh Borah

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