# Detection of *Mec-A* gene in CoNS Resistant to 3rd Generation Cephalosporins Isolated from Surgical Wound Infection

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**Abstract**

**Background:** The risk of nosocomial infection has been estimated as two to twenty times higher in developing countries than that of developed countries. Nosocomial infections in Bangladesh exceeds 30% in some Hospitals.

**Objective:** To Detect Mec-A gene in CoNS Resistant to 3rd Generation Cephalosporins Isolated from Surgical Wound Infection

**Methods:** This is a Cross-sectional type of descriptive study. The study population of this study includes adult Patients of different ages and both sexes suffering from nosocomial wound infections admitted in Surgery wards, Orthopaedic wards, Gynaecology and Obstetrics wards and Burn Unit of Rajshahi Medical College Hospital. The sample size is 330 and specimens of Surgical wound swabs are collected from the patients with surgical wound infections admitted into the Departments of General Surgery, Orthopaedic Surgery, Gynaecology and Obstetrics Surgery and Burn Unit. Wound swabs were collected from the leading edge of the wound showing signs of infections.

**Results:** A total of 330 different post-surgical wound swabs were obtained from various departments of RMCH Rajshahi for aerobic culture and sensitivity. Among these, culture yielded growth of 297 (90%) cases whereas 33 (10%) samples yielded no growth. In this study regarding antimicrobial resistance of Coagulase Negative Staphylococci, isolates show highest resistance to cefixime and ampicillin (64.58%), followed by ceftriaxone (60.42%), gentamycin (54.17%), ciprofloxacin and ceftazidime both were (52.08%).

**Conclusion:** This study focused that the incidence rate of CoNS in surgical wound infection should not be ignored. Rather their incidence is gradually increasing. On the other hand, CoNS have emerged as a multidrug resistant pathogen bearing mecA gene. So, this pathogen should be given more emphasis especially in dealing with surgical wound infection. Indiscriminate use of antibiotics prior to susceptibility testing should be stopped immediately. Efforts should also be made to enact regulations on antibiotic usage

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**Keywords:** CoNS, *mec-A* gene, 3rd generation cephalosporins, surgical wound infection

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## Introduction

It is quite obvious that patients are better treated in hospitals than anywhere else. Historically, hospitals have a notorious reputation for infection. Large number of sick under a single roof could easily facilitate the transmission of infectious diseases from patient to patient. Hence the term 'Nosocomial' or 'Healthcare associated infections' (HCAI) arises. Nosocomial or Hospital Acquired Infections are usually defined as infections occurring within forty-eight hours of hospital admission, three days of discharge or thirty days of an operation.[1] They affect 1 in10 patients admitted to hospital. Nosocomial infections occur worldwide and affect both developed and resource-poor countries. A prevalence survey conducted under WHO in 55 hospitals of 14 countries representing four WHO Regions (Europe, Eastern Mediterranean, South-East Asia and showed an average of 8.7% of hospital patients had nosocomial infections. The risk of nosocomial infection has been estimated as two to twenty times higher in developing countries than that of developed countries. Nosocomial infections in Bangladesh exceeds 30% in some hospitals. In 2011, a report from Dhaka Medical College Hospital shows 46.2% rate of nosocomial infections on surgical and burn units. While the vast majority of CoNS infections are characterized by a acute, subacute or even chronic course of infection, with a nonspecific, mild to aggressive, severe, and/or lethal infections have also been reported. However, data giving valid species identification are sometimes absent in these cases.[2]

Sufficient data is still not available in our country about the prevalence of CoNS, resistant patterns of their different species and treatment. Whereas CoNS species identification is necessary in order to establish epidemiological trends, to overcome treatment failure and to determine as an important cause of infection in hospitals.[3]

*Objective*

Detection of Mec-A gene in CoNS Resistant to 3rd Generation Cephalosporins Isolated from Surgical Wound Infection.

## Methods

This is a Cross-sectional type of descriptive study. The study population of this study includes adult Patients of different ages and both sexes suffering from nosocomial wound infections admitted in Surgery wards, Orthopaedic wards, Gynaecology and Obstetrics wards and Burn Unit of Rajshahi Medical College Hospital. All the sample and relevant information was collected from the patients with surgical wound infections and admitted into the Departments of General Surgery, Orthopaedic Surgery, Gynaecology and Obstetrics Surgery and Burn Unit of Rajshahi Medical College Hospital. Microbiological study was carried out in Department of Microbiology, Rajshahi Medical College. This study was conducted in January, 2018 to December, 2018 and the sample size was 330 specimens of Surgical wound swabs. The Sampling technique was Purposive sampling technique. The Data collection Instrument was Partially structured duly pretested data sheet. Informed written consent was taken from each patient.

## Results

A total of 330 specimens were collected from wound infection cases and cultured in different bacteriological culture media.

Table I: The frequency of bacterial isolates according to the place of specimen collection (n=330)

| Place of Specimen collection | Number of Samples | Culture positive | | Total Culture positive | Culture negative |
|---|---|---|---|---|---|
| | | Single isolates | Multiple isolates | | |
| General Surgery | 117 (35.45%) | 97 (29.39%) | 09 (2.73%) | 106 (32.12%) | 11 (3.33%) |
| Orthopedic Surgery | 97 (29.39%) | 77 (23.33%) | 07 (2.12%) | 84 (25.45%) | 13 (3.94%) |
| Gynecology & Obstetrics | 91 (27.58%) | 82 (24.85%) | 04 (1.21%) | 86 (26.06%) | 05 (1.52%) |
| Burn & Plastic Surgery | 25 (7.58%) | 19 (5.76%) | 02 (0.61%) | 21 (6.36%) | 04 (1.21%) |
| Total | 330 (100%) | 275 (83.33%) | 22 (6.67%) | 297 (90.00%) | 33 (10.00%) |

In Table I, Among 330 specimens, 117 (35.45%) from General Surgery wards, 97 (29.39%) from Orthopedic Surgery wards, 91 (27.58%) from Gynecology and Obstetrics wards and 25 (7.58%) from Burn and Plastic Surgery Unit. Among all, culture yielded growth were 297 (90.00%) cases and they are distributed as General Surgery 106 (32.12%), Orthopedic Surgery 84 (25.45%), Gynecology and Obstetrics 86 (26.06%) and Burn and Plastic Surgery 21 (6.36%) respectively.

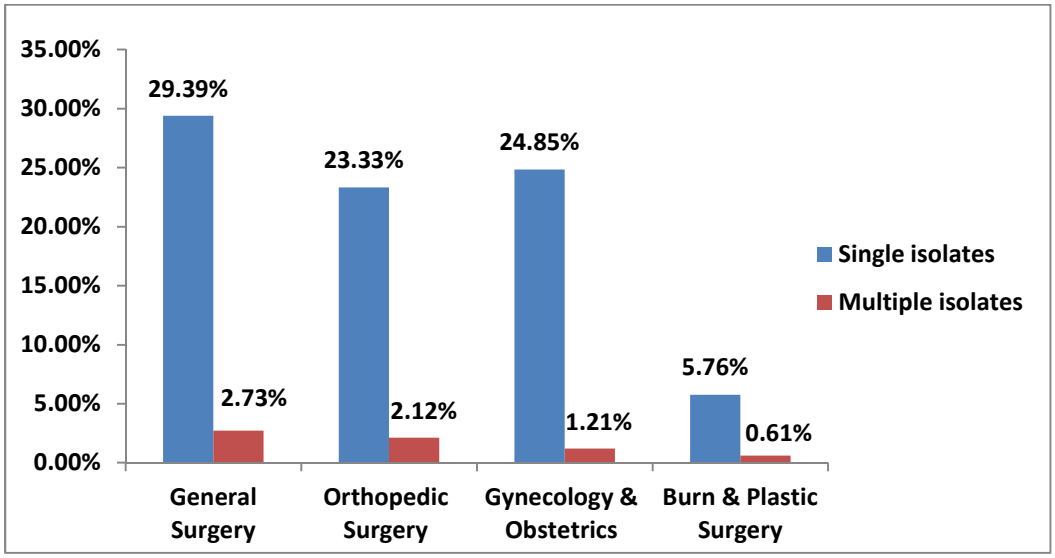


Figure 1. Frequency of single isolates and multiple isolates according to the place of specimen collection.

Among all samples, a total of 297 (90%) culture positive cases distributed as from General Surgery wards 97 (29.39%) were single isolates, 09 (2.73%) were multiple isolates. From Orthopedic Surgery wards 77 (23.33%) were single and 07 (2.12%) were multiple isolates. Then from Gynecology & Obstetrics wards 82 (24.85%) were single isolates and 04(1.21%) were multiple isolates. Finally, from Burn & Plastic Surgery unit 19(5.76%) were single isolates and 02 (0.61%) were multiple isolates respectively.

Table II: Age and sex distribution of wound infection cases. (n=330)

| Age (Years) | Number of samples | Male | Female | Culture-positive cases | Male | Female |
|---|---|---|---|---|---|---|
| 19-30 | 108 (32.73%) | 36 (10.90%) | 72 (21.82%) | 98 (29.70%) | 31 (9.39%) | 67 (20.30%) |
| 31-40 | 97 (29.40%) | 51 (15.45%) | 46 (13.94%) | 83 (25.15%) | 45 (13.64%) | 38 (11.52%) |
| 41-50 | 76 (23.03%) | 40 (12.12%) | 36 (10.91%) | 67 (20.30%) | 38 (11.52%) | 29 (8.79%) |
| More than 50 | 49 (14.85%) | 32 (9.70%) | 17 (5.15%) | 49 (14.85%) | 28 (8.48%) | 21 (6.36%) |
| Total | 330 (100%) | 159 (48.18%) | 171 (51.82%) | 297 (90.00%) | 142 (43.03%) | 155 (46.97%) |

Table-II shows age and sex distribution of study population. There were 4 age groups: (19-30) years, (31-40) years, (41-50) years, and >50 years. The minimum age was 20 years and the maximum was 70 years. Maximum 108 (32.73%) cases were male 36 (10.90%), female 72 (21.82%) were found within the age group of 19-30 years followed by 97 (29.40%) cases (male-15.45% & female-13.94%), then 76 cases (12.12%-male, 10.91%-female) and 49 cases (14.85%-male, 9.70%-female) were within the age group of 31-40 years, 41-50 years and above 50 years respectively. As a whole, males were 48.18% and females were 51.82% giving a male and female ratio 1:1.09. Highest number of culture positive cases 98 (29.70%) were seen in the age group of 19 to 30 years followed by 31 to 40 years 83 (25.15%), 41 to 50 years 67 (20.30%) and above 50 years of age group 49 (14.85%).

Table-III: Identified single species of bacteria from different clinical samples. (n=297)

| Identified bacteria | General Surgery Ward | Orthopedic Ward | Gynecology & Obstetrics Ward | Burn & Plastic Surgery Ward | Total isolates | |
|---|---|---|---|---|---|---|
| *S.aureus* | 37 (12.45%) | 28 (9.43%) | 30 (10.10%) | 8 (2.70%) | 103 (34.68%) | Gram Positive growth |
| CoNS | 14 (4.71%) | 19 (6.40%) | 10 (3.37%) | 5 (1.68%) | 48 (16.16%) | 151 (50.84%) |
| Gram Negative Bacilli | 55 (18.52%) | 37 (12.46%) | 46 (15.49%) | 8 (2.70%) | 146 (49.16%) | Gram Negative growth 146 (49.16%) |
| Total | 106 (35.69%) | 84 (28.28%) | 86 (28.96%) | 21 (7.07%) | 297 (100%) | |

Table III shows the identified single species of bacteria from different clinical samples. Out of 297 culture positive samples, *Staphylococcus aureus* was 103 (34.68%), CoNS were 48 (16.16%) and Gram-negative bacilli were 146 (49.16%). Among *S. aureus* 12.45% from General Surgery followed by 10.10% from Gyene & Obs., 9.43% from Orthopaedics and 2.70% from Burn and Plastic Surgery. Among CoNS highest 6.40% from Orthopaedics followed by 4.71% from General Surgery, 3.37% from Gyene & Obs and 1.68% from Burn and Plastic Surgery. Among gram negative bacilli highest 18.52% from General Surgery followed by 15.49% from Gyene & Obs 12.46% from Orthopaedics and 2.70% from Burn and Plastic Surgery.

Table IV: Pattern of mixed organisms isolated from post-surgical wound infection. (n=22)

| Combination of mixed organisms | General Surgery Ward | Orthopaedic Ward | Gynaecology & Obstetrics Ward | Burn & Plastic Surgery | No. of Combination |
|---|---|---|---|---|---|
| *Staphylococcus aureus* + Gram negative bacilli | 6 (27.27%) | 5 (22.73%) | 2 (9.09%) | 1 (4.55%) | 14 (63.64%) |
| *CoNS* + Gram negative bacilli | 3 (13.46%) | 2 (9.09%) | 2 (9.09%) | 1 (4.55%) | 8 (36.36%) |
| Total | 9 (40.91%) | 7 (31.82%) | 4 (18.18%) | 2 (9.09%) | 22 (100%) |

Table IV shows pattern of mixed growth from different clinical samples. Out of 297 culture positive samples, total 22 samples yielded dual growth. Mixed growth of *S. aureus* & Gram-negative bacilli was found in 14 (63.64%) cases. Out of which, 27.27% were from General Surgery, 22.73% from Orthopaedics, 9.09% from Gynaecology & Obs. and 4.55% from Burn & Plastic Surgery. Mixed growth of CoNS & Gram-negative bacilli was found in 36.36% cases. Out of which, 13.46% were from General Surgery, 9.09% from Orthopaedics, 9.09% from Gynaecology & Obs. and 4.55% from Burn & Plastic Surgery.

Table V: Identification of CoNS into common species. (n=48)

| Species | Novobiocin susceptibility | Urease test | Phosphatase test | Percentage |
|---|---|---|---|---|
| *S. epidermidis* | Susceptible | Positive | Positive | 29 (60.42%) |
| *S. haemolyticus* | Susceptible | Negative | Negative | 7 (14.58%) |
| *S. saprophyticus* | Resistant | Positive | Negative | 6 (12.50%) |
| Others | Variable | Variable | Variable | 6 (12.50%) |

Table V shows different species of CoNS found in post-surgical wound swabs. Here majority of the CoNS were *S. epidermidis* (60.42%) followed by *S. haemolyticus* (14.58%) and *S. saprophyticus* (12.50%). Unidentified CoNS species were also (12.50%).

Table VI: Antimicrobial resistant pattern of identified Coagulase Negative Staphylococci. (n= 48)

| Antimicrobial Agents | Sensitive | Intermediate Resistant | Resistant |
|---|---|---|---|
| Cefoxitin (30µg) | 36 (75.00%) | 1 (2.08%) | 11 (22.92%) |
| Ampicillin (30µg) | 15 (31.25%) | 2 (4.17%) | 31 (64.58%) |
| Amoxiclav (20/10µg) | 22 (45.83%) | 2 (4.17%) | 24 (50.00%) |
| Amikacin (30µg) | 24 (50.00%) | 2 (4.17%) | 22 (45.83%) |
| Gentamycin (30µg) | 19 (39.58%) | 3 (6.25%) | 26 (54.17%) |
| Ciprofloxacin (5µg) | 21 (43.75%) | 2 (4.17%) | 25 (52.08%) |
| Cefixime (30µg) | 16 (33.33%) | 1 (2.08%) | 31 (64.58%) |
| Ceftriaxone (30µg) | 18 (37.50%) | 1 (2.08%) | 29 (60.42%) |
| Ceftazidime (30µg) | 22 (45.83%) | 1 (2.08%) | 25 (52.08%) |
| Cefotaxime (30µg) | 22 (45.83%) | 2 (4.17%) | 24 (50.00%) |
| Imipenem (10µg) | 24 (50.00%) | 2 (4.17%) | 22 (45.83%) |
| Vancomycin (30µg) | 47 (97.92%) | 0 (0%) | 1 (2.08%) |

Table VI showing antimicrobial resistance pattern of Coagulase Negative Staphylococci. The isolates show highest resistance to Cefixime and ampicillin both were (64.58%) respectively, followed by Ceftriaxone (60.42%), Gentamycin (54.17%), Ciprofloxacin and Ceftazidime both were (52.08%). The highest sensitivity was shown against Vancomycin (97.92%) followed by Cefuroxime (68.75%), Imipenem (64.58%), and Novobiocin were (56.25%).

Table VII: Frequency of MRSA and MRCoNS isolates based on Cefoxitin resistance

| Isolates | No. of Organism |
|---|---|
| MRSA (n=103) | 47 (45.63%) |
| MRCoNS (n=48) | 11 (22.92%) |

Table-VII shows frequency of Methicillin Resistant *Staphylococcus aureus* (MRSA) and Methicillin Resistant Coagulase Negative Staphylococci (MRCoNS) based on Cefoxitin resistance. Out of 103 *S. aureus* isolates, 47 (45.63%) were Methicillin resistant. Whereas among 48 CoNS isolates, 11 (22.92%) were MRCoNS.

Table VIII: Distribution of *mecA* gene among different CoNS. (n=48)

| Name of species | *mecA* positive isolates | Total isolates |
|---|---|---|
| *S. epidermidis* | 5 (10.47%) | 29 (60.42%) |
| *S. haemolyticus* | 1 (2.08%) | 7 (14.58%) |
| *S. saprophyticus* | 0 (0%) | 6 (12.50%) |
| Others | 1 (2.08%) | 6 (12.50%) |
| Total | 7 (14.58%) | 48 (100%) |

Table VIII shows distribution of *mecA* gene among different CoNS. Out of 29 *S. epidermidis,* 5 found to carry *mecA* gene. Whereas 1 of *S. Haemolyticus* and 1 unidentified species were *mecA* positive isolate.

**Discussion**

A total of 330 different post-surgical wound swabs were obtained from various departments of RMCH Rajshahi for aerobic culture and sensitivity. Among these, culture yielded growth of 297 (90.00%) cases whereas 33 (10%) samples yielded no growth. This study is more or less similar with the study of Negi *et al,* and Laxshmi N *et al,.* which were 93.3% and 93% respectively. But the rate is dissimilar with the study of Begum *et al*, Yaqub *et al,.* 2018 and Bastola R *et al,.* and their isolation rates were 61.8%, 67% and 48.6% respectively.[4] This low culture positivity might be due to consumption of antibiotics prior to sample collection. The culture negative cases were 33 (10%). It might be due to samples were collected from sites where there was no organism or there was presence of anaerobic bacterial infection. Among all culture positive cases, mono-microbial growth was 275 (83.33%) and poly-microbial growth was 22 (6.67%).[5] This corresponds with the study of Nahar S G *et al,* in Bangladesh found single isolates 87.4%, multiple isolates 8.4%; Negi *et al,.* 2015 in India found single isolates 91.2%, multiple isolates 5.1%; and Saaiq *et al,* in Pakistan found 92.6% single isolates & 7.4% multiple isolates. In this study, *S. aureus* is the most common isolates 103 (34.68%). Our study is similar with the study of Nahar *et al,*, in Bangladesh, Sharma *et al,* in India and Asres G *et al,.* in Ethiopia which were 32.4%, 35% and 33.3% respectively. This finding is dissimilar with B. Ananthi *et al,* in India, Roy S *et al* in Bangladesh and Upreti in Nepal

which were 26.1%, 55.7% and 47.1% respectively.[6] Higher isolation rate of *S.aureus* in this study may be their prevalence in the hospital is more, improper personal hygiene and inappropriate hospital sanitation. In this study regarding antimicrobial resistance of Coagulase Negative Staphylococci, isolates show highest resistance to cefixime and ampicillin (64.58%), followed by ceftriaxone (60.42%), gentamycin (54.17%), ciprofloxacin and ceftazidime both were (52.08%). The resistance pattern of cefixime was almost similar with Nadakuduru *et al* and Seng R *et al* which were 65.31% and 68.30% respectively.[7] But cefixime resistance found lower in Charrakh A H *et al* and Asres G *et al* were 40.00% and 35.10% respectively. It might be due to selective pressure by cefixime as it is routinely prescribed.The resistance pattern of Ampicillin found almost similar with Rahman M S *et al* and Kanwalpret *et al* which were both 66%. But did not correspond with Begum S *et al* and Beukes LS *et al* which were much higher as 72.50% and 82.80% respectively.[8] This dissimilarity might be due to smaller sample size in these studies. Ceftriaxone resistance was found 60.42%. Similar resistance was found by Nahaei *et al* and Latika S *et al* which were 66.00% and 60.00% respectively.[9] This increased resistance may be due to extensive use of 3rd generation cephalosporin and other beta lactam drugs injudiciously. In this study, cefoxitin susceptibility was found 75% which was more or less similar with Kanwalpret *et al* and Ferreira A M *et al,* which were 79% and 73.2% respectively. But this study is dissimilar with Beukes LS *et al*, Seng R *et al,* and Begum S *et al,* which were 14.29%%, 26.90% and 50% respectively. Regarding amikacin susceptibility, we found 50.00% isolates were susceptible. This is more or less similar with the study of Kanwalpret *et al,* and Begum *et al* which were 43.84% and 48.57% respectively. But dissimilar with the study of Anathi *et al* and Mantravedi et al which were both 100%. In this study, out of 103 *S.aureus* isolates, 47 (45.63%) were identified as MRSA by cefoxitin disc test.[10] This is similar with the study of Mohammad N *et al*, Dutta S *et al,.* 2013 and B Shrestha *et al,*which were 44.44%, 46% and 45%. But the finding is dissimilar with Shamsuzzaman *et al*, Islam T *et al* and Bastola R *et al* which were 34%, 65.15% and 58.3% respectively. This higher isolation rate of MRSA was might be due to using oxacillin disc instead of cefoxitin disc. Regarding MRCoNS, we found 22.92% of CoNS isolates were Methicillin resistant.[11] This finding is similar with Raghav *et al,* and Bhat P *et al* which were 26.93% and 24% respectively. But this finding is quite dissimilar with the findings of Ibadin *et al*, Nagasrilata B *et al* and Mart *et al* which were 46.30%, 78.19% and 83.84%. These higher isolation rate of MRCoNS is may be due to using oxacillin disc instead of cefoxitin disc.[12]

This study focused that the incidence rate of CoNS in surgical wound infection should not be ignored. Rather their incidence is gradually increasing. On the other hand, CoNS have emerged as a multidrug resistant pathogen bearing *mecA* gene. So, this pathogen should be given more emphasis especially in dealing with surgical wound infection.

*Conclusion*

CoNS are the normal flora of the human skin and mucous membrane. Recently it has got attention as a potential pathogen especially for nosocomial infections. In our study we found that frequency of CoNS infection and their resistance is increasing day by day. A large proportion of Bangladeshi people live below the poverty line and they are unable to complete the treatment course especially in case of more expensive antibiotics such as 3rd generation cephalosporin. On the other hand due to arbitrary use of antibiotics, common

pathogens like CoNS develops resistance against frequently used drugs. Negligence of infection worsens the condition and makes treatment complicated to unaffordable. In most cases, patients initially take antibiotic without consulting an expert. As a result, microorganisms get suboptimal pressure at which they are not killed rather they acquire their ability to withstand the antibiotic pressure and emerge as antibiotic-resistant strain. Indiscriminate use of antibiotics prior to susceptibility testing should be stopped immediately. Efforts should also be made to enact regulations on antibiotic usage.

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