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Epidemiology, Antimicrobial susceptibility patterns and outcomes of bacteremia in an Apex trauma center of a tertiary health care institute with special reference to Methicillin Resistant *Staphylococcus aureus* (MRSA): A Prospective Cohort study

[1]Dr.Sangram Singh Patel, [2]Nidhi Yaduvanshi, [3]Dr.Chinmoy Sahu, [4]Dr.Sweta Singh, [5]Prof.Amit Agarwal, [6]Prof.Ujjala Ghoshal

[1]Assistant professor, [2,4]Senior Resident, [3]Associate professor, [5]Professor, [6]Professor & Head
[1, 2, 3, 4, 6]Department of Microbiology, SGPGIMS, Lucknow, U.P. India
[5]Endocrine Surgery and Chief Medical Superintendent, SGPGIMS,Lucknow,U.P.,India

Corresponding Author
Dr.Sweta Singh
Senior Resident, Department of Microbiology, SGPGIMS, Lucknow, U.P. India

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ABSTRACT

Background: Bacteremia in trauma patients is a significant cause of mortality, increased hospital stay and overall cost.It can be caused by both Gram negative as well as Gram positive pathogens.*Escherichia coli*,*Klebsiella pneumoniae* and *Staphylococcus aureus* are the predominantly isolated organisms.Both the group of pathogens are associated with significant mortality rates.

Aims: This study was planned to study the demographic parameters, profile of antibiotic sensitivities, molecular characterization of resistance mechanisms,as well as follow up of the patients admitted to our trauma centre.

Methodology: The current study was undertaken for a period of one year from January 2018 to December 2018 and 2 blood samples (1 set) were collected from each patient ;one in Aerobic Bactec and the other in Anaerobic Bactec bottle.Further processing was done in the microbiology laboratory as per standard guidelines.

Results: The Positivity rate of 15 % was seen in the collected blood samples. *Klebsiella pneumoniae* (37%) was the most predominant isolate while ;MRSA (29%), *E.coli* (15%) and *Acinetobacter baumanii* (9%) were among the other common isolates. Colistin was found to be the most potent antibiotic for Gram negative bacilli; whereas Vancomycin and Teicoplanin proved highly efficacious for Gram positive ones.Mec-A genes were isolated from majority of *MRSA (Methicillin resistant staphylococcus aureus*) isolates and MCR-1 from colistin resistant isolates.

Conclusion: Bacteremia along with other invasive procedures such as central line insertion or catheterization are associated with increased mortality rates in trauma patients.This important aspect needs to be studied in greater detail for better patient management and outcomes.

Keywords: Antibiotic – Trauma- Anaerobic-Vancomycin -Teicoplanin - Colistin

INTRODUCTION

Trauma care forms an essential part of a health care system. Trauma related deaths show a tri-modal distribution. Injury to the great vessels or the heart is usually non-salvageable and is the first spike. Second spike occurs in the first 6 hours and leads to early deaths .Finally, deaths which occur late and are due to sepsis and multiorgan failure. [1]

Bacteremia is an important and significant cause of morbidity and mortality in trauma patients due to the breach in defence mechanism of skin [2] The risk

also increases due to the various invasive procedures performed in these patients during their hospital stay.[3,4] Blood stream infections (BSI) increase the length of stay, risk of in-hospital infections and overall cost. Overproduction of inflammatory mediators,downregulation of cell mediated immunity along with cytokine surge leads to complications in trauma patients.[5,6] BSIs can be community acquired or nosocomial in nature.Nosocomial BSIs are associated with a higher mortality rates as compared to community ones.The mortality usully ranges from 12% to 80%.[7] The source of origin of BSIs can be primary or secondary in type. Primary BSI originates from the intravascular or cardiovascular system itself; while, secondary BSI has some other infectious focus .The causative organisms of BSI can be both Gram negative as well as positive .Among the Gram positives ;*Staphylococcus species* are the most commonly isolated ones. [8]On the other hand;*Pseudomonas aeruginosa, Klebsiella pneumonia, Escherichia coli* and *Enterobacter spp*. predominate among the Gram negative isolates.[9] The mortality and fatality rates varies among species.Staphylococcus aureus has a high mortality rates varying from 7- 39%.[10] Even Gram negative bacteremias account for a large no. of mortality cases ranging from 6- 42%.[11,12]

There is sparse data on the causes of bacteremia as well as their sensitivity patterns in hospitalized trauma patients from India.Hence, the current study was planned to study the demographic parameters ,antibiotic sensitivity profiles, molecular characterization of resistance mechanisms, as well as follow up of these trauma patients.

MATERIALS AND METHODS:

Study Setting & Design: This study was an observational, prospective cohort study and undertaken for a period of one year from January 2018 to December 2018 at Apex trauma centre of our tertiary health care institute.

Inclusion Criteria: Patients who were admitted for a minimum of 2 days and showing suspected signs of sepsis were included in the study.

Sample Collection and Transport:

According to our Institute's protocol; 2 blood samples (1 set) were collected from each patient ;one in Aerobic Bactec and the other in Anaerobic Bactec bottle. All samples were transported to the department of Microbiology immediately. They were further kept in automated BacT/ALERT system (bioMieréux, Durham, USA) and further processing was done as per the manufacturer's instructions. [13,14]

Sample Processing:

A. Culture: Specimens were cultured on both MacConkey and blood agar plates according to standard microbiological techniques. Further,colonies were isolated and subcultures done accordingly.

B. Identification:

1. **Routine biochemical testing:**

   Routine staining and biochemical tests were employed for preliminary identification of the isolates in our laboratory.[14]

2. **Automated identification :** MALDI TOF MS and Vitek 2 system (Biomerieux, France) were used for final confirmation of the identity of the isolates[15]

C. Resistance Detection Method

1. Disc Diffusion Test: Kirby- Bauer's disk diffusion method on Muller Hinton agar were used for antibiotic sensitivity testing and interpreted based on Clinical and Laboratory Standards Institute (CLSI) guidelines. [16]

2. Minimum inhibitory concentration (MIC): MICs were further recorded as per CLSI guidelines for the following drugs: Vancomycin , Linezolid for Gram positive cocci and Colistin, Tigecycline for Gram negative bacilli respectively.

3.Genotypic Method: Colistin resistant isolates were further tested for presence of mcr-1 gene as the plasmid-mediated spread of this colistin resistance gene poses a significant public health problem.[17] MRSA isolates were also tested for mec genes using PCR amplification.[18]

Patient follows up:

Various demographic profiles and clinical findings of the trauma patients were kept in computer database for follow up and outcomes. Risk factors along with mortality rates were also assessed.

Statistical Analysis

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Social Sciences software for Windows Version 14.0 (SPSS, Inc., Chicago, IL, USA) was used for statistical analysis. Enumeration data were expressed as percentage values and sensitivities were calculated by Graph Pad Prism Software.

RESULTS

A total of 1123 blood samples with clinical suspicion of BSI were received in the laboratory. Of these; 171 (15.2%) showed growth of pathogens and the rest were either sterile or contaminated with more than two different types of bacterial growth.

The demographic parameters were also studied with all the possible risk factors. (Table 1). Males comprised of the majority of trauma cases (81%) as compared to females.Median age of the bacteremia patients were around 40 years with the range from 20-72 years. 21 % of the cases gave positive history of hospital stay within the last 30 days wheras;51% were transferred patients from other peripheral hospitals.On assessment of the possible risk factors ;central line insertion was seen in approx.63% of the cases , mechanical intubation in 47% and urinary catheter was placed in 37% of the patients.32% of the patients had significant antibiotic exposure before admission.

The predominant pathogen in these cultures were gram negative bacilli in 96 (56.4 %)cases while rest(75,43.8%) being Gram positive cocci. *Klebsiella pneumoniae* (37%) was the predominant isolate ; MRSA (29%), *E.coli* (15%) and *Acinetobacter baumanii* (9%)were the other common pathogens. (Fig 1) .Minority of the samples were also positive for *MSSA (Methicillin sensitive staphylococcus aureus), MRCONS(methicillin resistant coagulase negative staphylococcus species),Enterococcus* spp among gram positive cocci and ,*Enterobacter cloacae,Pseudomonas aeruginosa* among Gram negative ones. Detailed antibiotic sensitivities are mentioned separately for Gram negative and positive isolates in Fig 2 &3.

The Prevalence of MRSA was seen as higher among the trauma patients (29%)as compared to the routine isolation from wards (18%). Colistin (98%) followed by imipenem (47%) and meropenem (38%) were the most effective drugs for gram negative bacilli.(Table 2). Cefoperazone-sulbactam (19%) ,Gentamicin (8%) and piperacillin –tazobactam (3%) showed moderate sensitivity.Teicoplanin (100%), vancomycin (100%) followed by clindamycin (65%) showed efficacy against MRSA isolates. *VRE (Vancomycin resistant enterococci)* was seen in 2 cases only. The other Gram positive isolates also showed good sensitivity to the above three drugs and moderate action was noticed for amikacin,levofloxacin and erythromycin.All the MRSA isolates were detected by cefoxitin disc method. 98% isolates were positive for *mecA* gene and only one (2%) was positive for *mecC* gene by PCR analysis. (Fig 4) Colistin resistant isolates were also tested for MCR-1 gene and positivity was seen in all the resistant isolates.

On an average only 1 blood culture set was obtained from each patient in Gram negative bacteremia ; whereas 2 sets were obtained in some cases of Gram positive cases.More than 2 episodes of bacteremia were noticed in almost 48% of the cases.The average duration of hospital stay was studied as 33 days with the range of 16-42 days and the mean duration of development of bacteremia was approx.16 days from the time of admission.More than one organism or polymicrobial growth was noticed in overall 13% patients.The overall mortality was seen in 21% of the bacteremia cases with Gram negative being slightly on the higher side as compared to Gram positive ones (23% vs 19%).

DISCUSSION

Very few literatures have been published in the past highlighting the importance of bacteremia in trauma patients.The current study is one such approach to study the different causes of bacteremia along with the possible risk factors in such patients. Ours is a tertiary care institute with a muti-bedded apex trauma centre which caters to the needs of a large population group of our state as well as the neighbouring ones. Trauma patients are at a higher risk due to the hematological and immunological changes as compared to other routine inpatient admissions. [19] Trauma patients also experience a longer hospital stay along with increased chances of nosocomial infections and greater mortality rates.

Various risk factors for bacteremia assessed in our study found central line insertions and intravenous catheters as an important risk factor.This has also been studied as an important risk factor by a U. S. based study in similar patients.[20] Prior antibiotic

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exposure and inappropriate use of empirical antibiotics has also been proved to be associated with increased mortality in trauma patients.[21,22] Mechanical intubation has also been seen as an independent risk factor in such critically ill patients.[22]

Our study showed predominance of Gram negative isolates in bacteremia cases mainly Klebsiella and E.coli.This finding is very similar to the study conducted by Villegas et al. and Diakema et al. in ICU trauma patients.[23,24] They also reported E. coli in 11.8% and Klebsiella in 32.6% cases.These findings suggest a very pivotal role of Gram negative pathogens in causing bacteremia in trauma patients.

Previous literature search says a lot about the Staphylococcal bacteremia and the high mortality rates associated with it.In our study,MRSA was seen in almost 29 %of the cases while MSSA comprised of only a minority of <2% of the cases. Mec-A was positive in majority of the cases.This finding is very well in concordance with various past studies done by Forsblom *et al*.[25] , Conterno *et al*.,[26] and Kanafani *et al*.[27] .They assessed a range of 7% to 39% of Staphylococcal bacteremia in trauma cases. Our trauma centre reported much higher cases of MRSA than previously reported in other studies. A study done at trauma centre of AIIMS, Delhi reported 59% of MRSA cases in trauma patients. [28] Some other studies done by Parameswaran *et al*.[8] found MRSA in 26.7% of patients with CRBSI and Wisplinghoff *et al*.[20] found 41% of their strains were MRSA. The increase in our MRSA rates may be due to the increase in Methicillin rates over these years in various parts of the world ,especially in a developing country like ours.

The CONS isolates in our study were all reported to be methicillin resistant and were isolated from the patients only single time in both aerobic as well as anaerobic vials . This made the correlation more clearer and supported the fact that single time isolation of CONS should not be regarded as environmental contaminant .Rather;it should be correlated clinically for presence of sepsis or bacteremia;if any.Previous literature also supports the finding of single time isolation of CONS as being significant. Studies by Tak et al . [28] and Favre *et al*.[29] reported a higher mortality in patients with single blood culture positive for CONS as compared to patients with multiple blood cultures positive .

Our study detected only a few cases of VRE bacteremia and it was also not associated with significant mortality rates.This finding was very similar to the study done at AIIMS trauma centre on Enterococcal bacteremia which also reported very few VRE isolates. [30] A similar finding was suggested by Cho et al.; who also showed that VRE isolation was not associated with increased mortality rates. [31] Polymicrobial growth in blood culture bottles was seen in approx.11% of the cases.Higher frequency of polymicrobial growth was also seen in a similar study by Reigedas et al.[32]

Comparing the overall mortality rates among various studies in bacteremic trauma patients revealed the range of 18-39%.[20,33] Our study also revealed mortality rate of 21% which is well within this range.

Patients admitted to the trauma centres have compromised hemodynamic as well as immunologic status.The outcome in these patients usually depends on the level of injury,compromise of vascularity and infections at the site of trauma.Hence,attributing mortality only to bacteremia causes might be somewhat biased decision.

CONCLUSION

Gram negative and positive causes of bacteremia play a very significant part in determining further course of action in trauma patients.They are associated with increased hospital stay,increased mortality rates as well as cost of stay.Hence,it is of utmost importance on the part of microbiologists to timely diagnose the cause of bacteremia and guide the intensivist/clinician for proper institution of antimicrobial therapy.This can prove to be very vital and crucial in improving the patient outcomes as well as decrease the mortality rates.More studies on trauma patients from different centres are needed to further our knowledge on this important aspect of hospital settings.

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Ethical considerations

Informed consent was obtained from all the patients and their legal guardians(in case of minors) regarding the publication of images and clinical

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information in the journal.They were informed of the confidentiality of tha data ,however ,the anonymity cannot be guaranteed.

Research quality and ethics statement:

The authors of this manuscript declare that this scientific work complies with reporting quality and formatting and reproducibility guidelines set forth by the EQUATOR Network. The authors also attest that this clinical investigation was determined to require the Institutional Review Board/Ethics Committee review, and the corresponding protocol/approval number is IEC Code: 2018-56-IMP-103

Ethical conduct of research:

This study was approved by the Institutional Review Board / Ethics Committee. The authors followed applicable EQUATOR Network (http://www.equator-network.org/) guidelines during the conduct of this research project.

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Table 1: Demographic and clinical parameters in the admitted trauma patients

Variables	**Gram negative bacteremia (n=96, 56.4%)**	**Gram positive bacteremia (n=75 , 43.8%)**
Age,median (years)	42(20-68)	38(18-72)
Males	78	85
Females	22	15
Any hospital stay in last 30 days	18(18.7%)	26(34.6%)
Referral from other peripheral centres	54(56.2%)	48(64%)
Central line insertion	62 (64.5%)	65(86.6%)
Mechanical intubation	46(47.9%)	48(64%)
Urinary catheter insertion	38(39.5%)	36(48%)
Prior antibiotic exposure	29(30.2%)	34(45.3%)
No. of Positive Blood culture sets	1(1-2)	2(1-3)
Episodes of bacteremia>=2	41(42.7%)	56(74.6%)
Polymicrobial bacteremia	13(13.5%)	9(12%)
Days from admission to onset of bacteremia	16.5(7-26)	20(9-31)
Total duration of hospital stay	28(18-38)	29(16-42)
Mortality rates	23(23.9%)	19(25.3%)

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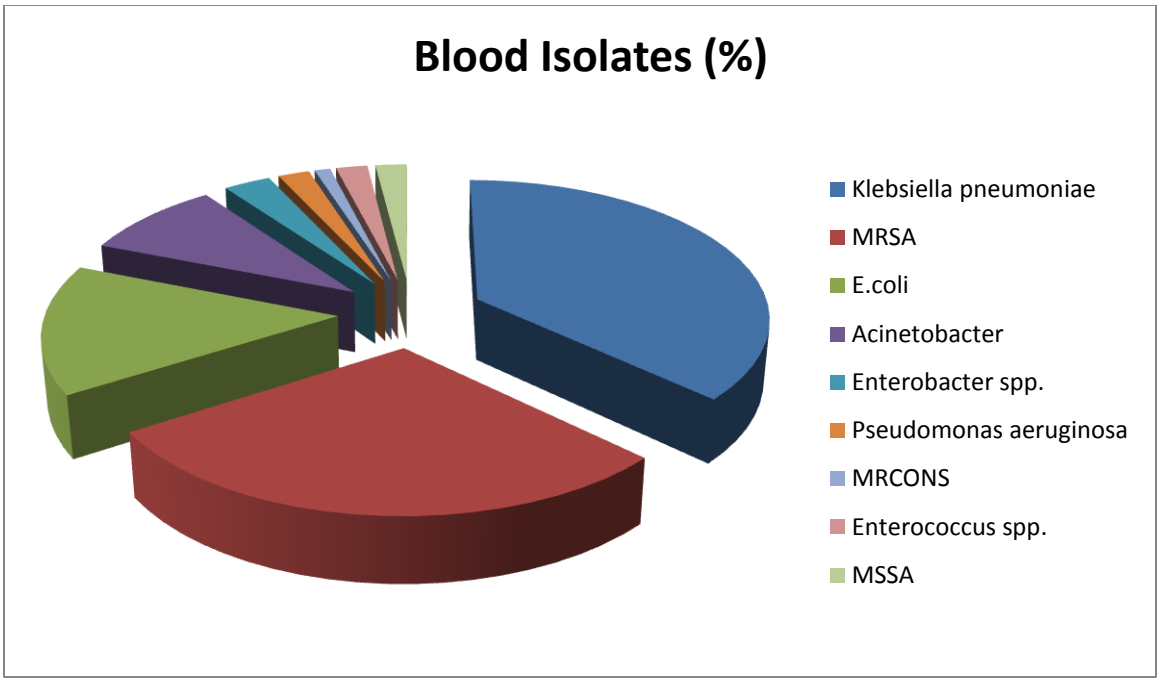

Fig 1: Distribution of various blood isolates

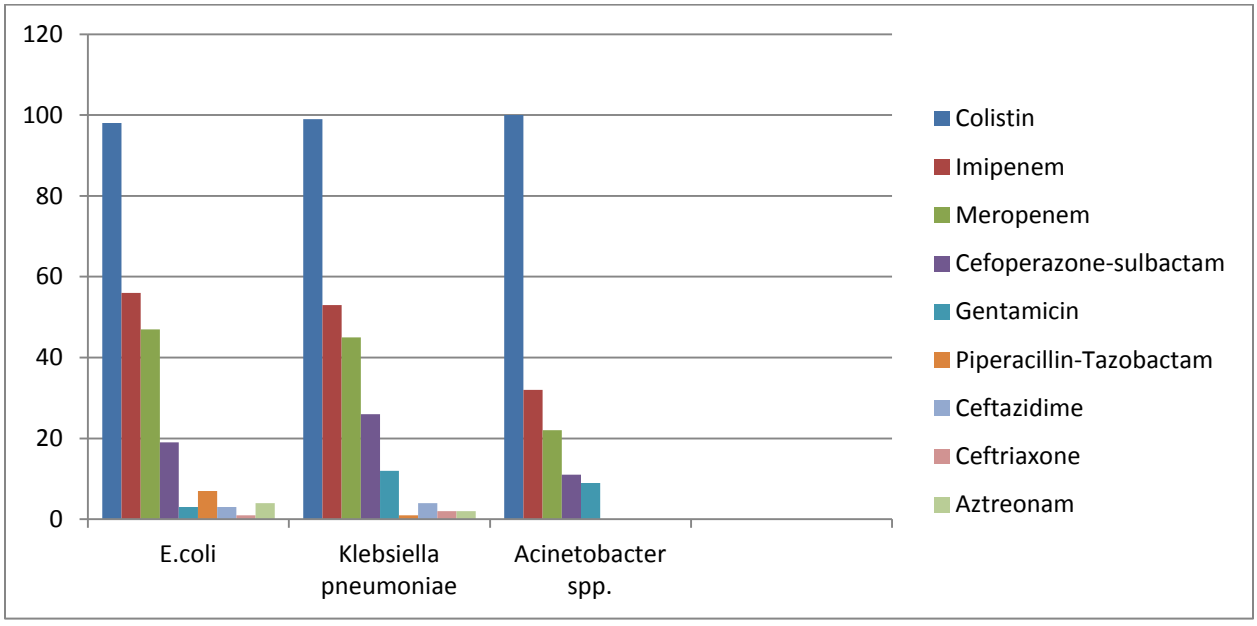

Fig 2: Sensitivity pattern in E.coli,Klebsiella, and Acinetobacter spp. to various first line drugs.

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Table 2: Sensitivity pattern in E.coli,Klebsiella, and Acinetobacter spp. to the three major drugs :Colistin,Imipenem and Meropenem (% sensitive)

Antibiotics	E.coli(%)	Klebsiella pneumonia(%)	Acinetobacter spp.(%)
Colistin	98	99	100
Imipenem	56	53	32
Meropenem	47	45	22

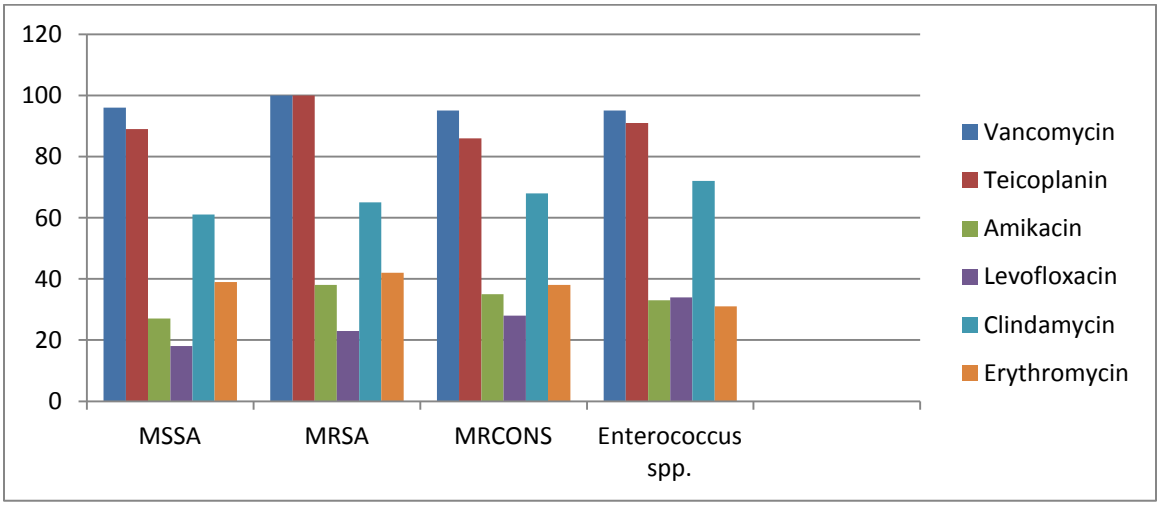

Fig 3: Sensitivity pattern in MRSA, MRCONS and Enterococcus spp. to various first line drugs.

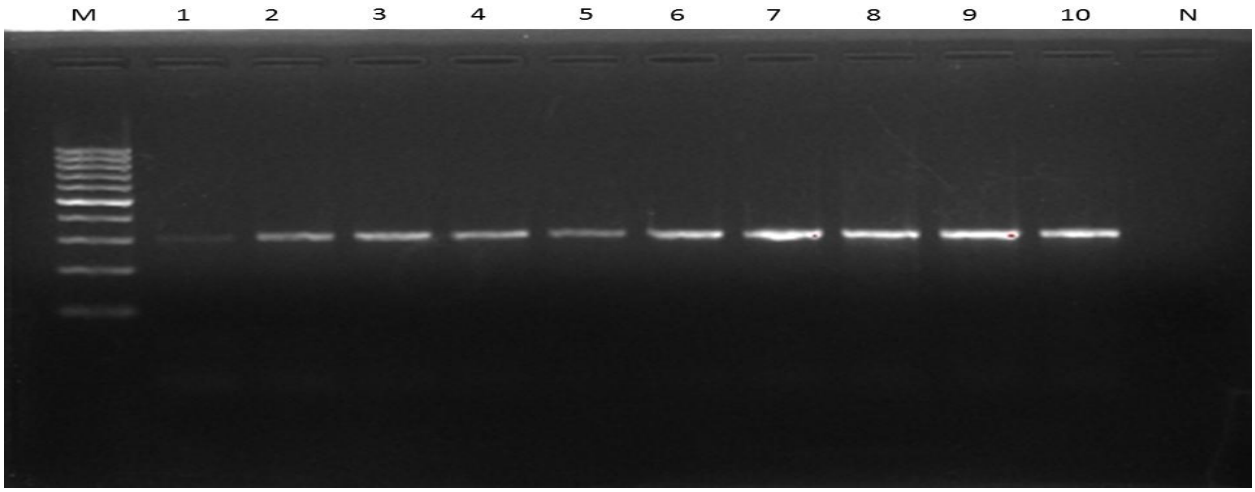

Fig 4: PCR showing mec-A gene in MRSA isolates of blood.

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