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The Antimicrobial Resistance of Staphylococcus Haemolyticus Isolated from Patients in Taif, Saudi Arabia

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Abstract **Original Research Article**

***Background*:** The spread of antibiotic-resistance genes in ecosystems has led to the emergence of antibiotic- resistant bacteria, leading to various antibiotic-resistant diseases worldwide. ***Materials and methods*:** This study aimed to characterize mute-drug resistant Staphylococci isolates from urine and wounds. We obtained 400 bacterial isolates that were tested for their pathogenicity through cultivation on blood agar, Vitek instrument was used for characterization of different antibiotic sensitivity, while PCR was used to detect the resistance gene. Excel and SPSS were used to analyze the data. All samples were obtained after institutional ethics review and participant's-consents ***Result*:** Of 400 160=40% females, 60%=240 were males) isolates were carried out from. Out of 400 twenty-one were beta-hemolysis almost of them were described as extensive Multi-drug resistant isolates were 4(21) as they resist 10 antibiotics, 1(21) resist nine antibiotics, 7(21) resist eight antibiotics, 3(21) against seven, 2(21) six antibiotics, 2(21) resist five antibiotics,1(21) resist four antibiotic and 1(21) resist two antibiotics. These twenty-one were positive in mecA gene amplification through PCR technique but negative for the Vancomycin resistance gene. ***Conclusion*:** There are high mecA genes among clinical isolates in our study further studies with a high sample size Staphylococcus haemolyticous is recommended.

Keywords: *Staphylococcus haemolyticus*, mecA gene, Vanc gene, MDR gram-positive.

INTRODUCTION

The clinical importance of coagulase-negative staphylococci is getting attention in the last few years (Hitzenbichler *et al*., 2017) (Szemraj *et al*., 2023) Coagulase-negative bacteria rarely cause urinary tract infections in children (Megged, 2022). Bailey. R found six episodes of coagulase negative during 21 months in clinics with 16.7% of all urinary tract infections (Bailey, 1973). Ahmed et.al found that the prevalence of coagulase-negative methicillin resistance in diabetic foot burns, and the abscess was 11.5, 10.3, and 15.6 respectively (Ahmed *et al*., 2021). Almjid *et.al*., (2020) assessed the incidence, types, risk factors, discovered organisms, and outcomes of surgical wound infections (SWIs) following heart surgery and demonstrated that Staphylococcus aureus, which is methicillin-susceptible, was isolated most of the time (45%), followed by Klebsiella and Pseudomonas species. CoNS is considered a skin contaminant in most clinical microbiology laboratories (Hagler & Dobkin, 1990)(Kline & Lewis, 2016). A Tanzanian study demonstrated that coagulase-negative staphylococci are the second causative agent of gram-positive that cause community types of urinary tract infections (Silago *et al*., 2022). Here we isolated the CONS in urine and wound samples with VITEK and subsequent tests of resistance strains with a polymerase chain reaction.

MATERIALS AND METHODS

Study Setting and Ethical Approval

A cross-sectional study was done in Taif city during 2021, the study was approved with ethical approval committee at UM-Alqura university faculty of applied health sciences, with written informed consent from all patients.

Samples Collection

Samples were collected from patients infected with urinary tract and from wound sites in different body parts. All samples were collected at King Abdulaziz Specialist Hospital in Taif City, Saudi Arabia. Transfer of samples was done immediately in sterilized containers and saved at 4^{O}C for microbial investigations.

Bacterial Isolations

The bacteria were isolated in different media as follows, nutrient agar was used as primary media for all isolates, and mannitol salt agar medium for staphylococcus species. All medium was incubated at 37 ^{O}C for consecutive three days. The isolation and microbial sensitivity were carried out with the VITEK2 system. We applied seventeen antibiotics for beta haemolytic staphylococcus which include (linezolid, teicoplanin, vancomycin, tetracycline, Fosfomycin, fusidic acid, mupirocin, rifamycin, trimethoprim/ sulfamethoxazole, cefoxitin screen, oxacillin, levofloxacin, moxifloxacin, inducible clindamycin resistance, erythromycin, and clindamycin).

DNA Extraction

DNA (genomic) was extracted with alkaline methods (Khedr *et al*., 2017). Briefly, 1.5 ml from the culture was taken and centrifuged immediately at 8000 speeds for one minute, the bacterial pellet was stored, then 250 µl of solution A was carefully added and well-mixed, then 250 µl from solution B was added and well-mixed, in addition, 250 µl of solution C was added with well-mixed as above steps, furthermore, centrifugation of overall mixer was done for all components at 13000 for five minutes. In the last step, the upper layer was removed and DNA was extracted as a pellet, 25 µl of each sample was added to a 5 µl loading buffer in an Eppendorf tube.

Polymerase Chain Reaction PCR for Meca and Vanc Gene

The polymerase chain reaction was done with two primers for mecA gene a) 5-AAAATCGTGGTAAAGGTTGGC-3 and b) 5 AGTTCTGCAGTACCGGATTTGC-3. The PCR steps were done with denaturation at 94^{0} C for five minutes, an annealing stage at 52^{0}C for one minute, extension step at 72^{0}C for three minutes. The final step of the extension was 15 minutes. The product was run in 1% agarose gel at 112 voltages and then finally the gel was stained with ethidium bromide for 30 minutes and showed with UV light. The Vanc gene was done with the same steps the difference was primers with the following sequence a) 5-ATGAATAGAATAAAAGTTGCAATAC-3b) 5-CCCCTTTAACGCTAATAATACGAT-3 the denaturation was done for three minutes, extension at 70.1^{0}C.

RESULTS

Of 400 160=40%females, 60%=240were males) isolates were carried out from. Out of 400 twenty-one were beta- hemolysis almost of them were described as extensive Multi-drug resistant isolates were 4(21) as they resist 10 antibiotics, 1(21) resist nine antibiotics, 7(21) resist eight antibiotics, 3(21) against seven, 2(21) six antibiotics, 2(21) resist five antibiotics,1(21) resist four antibiotic and 1(21) resist two antibiotics. These twenty-one were positive in mecA gene amplification through PCR technique but negative for the Vancomycin resistance gene. These twenty-one were positive in mecA gene amplification through PCR technique but negative for the Vancomycin resistance gene. The most effective one against tested twenty-one isolates was Nitrofurantoin, which eliminates all tested isolates, followed by five antibiotics Linezolid, Teicoplanin, Vancomycin, Fosfomycin, and Cefoxitin screen which eliminates all isolates except one only, then Mupirocin that eliminate all isolates except two isolates only were resistant against it. Fusidic Acid was effective against all isolates except three isolates, Clindamycin and Rifamycin eliminated all except six isolates, and Moxifloxacin was effective against all 7 isolates. Trimethoprim/Sulfamethoxazole was effective against twelve isolates out of test 21, then Erythromycin, Tetracycline, and Levofloxacin with bactericidal activity against seven isolates, Clindamycin was bactericidal against two only (figure2). All twenty-one isolates were positive for the MecA gene with partial amplification reaching 533bps as visualized on agarose gel electrophoresis (Figure 3). None of the twenty-one isolates has a Vancomycin-resistant gene as their PCR was negative without product**.** Agarose gel electrophoresis was carried out against the DNA ladder and showed negative amplicons as shown in Figure (4).

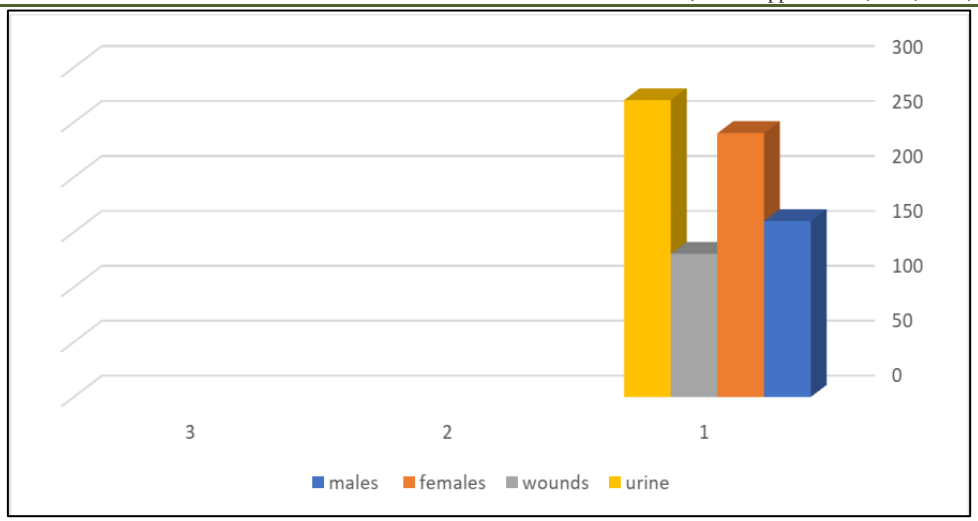

Fig 1: Socio-demographic characteristics and sample types in the study

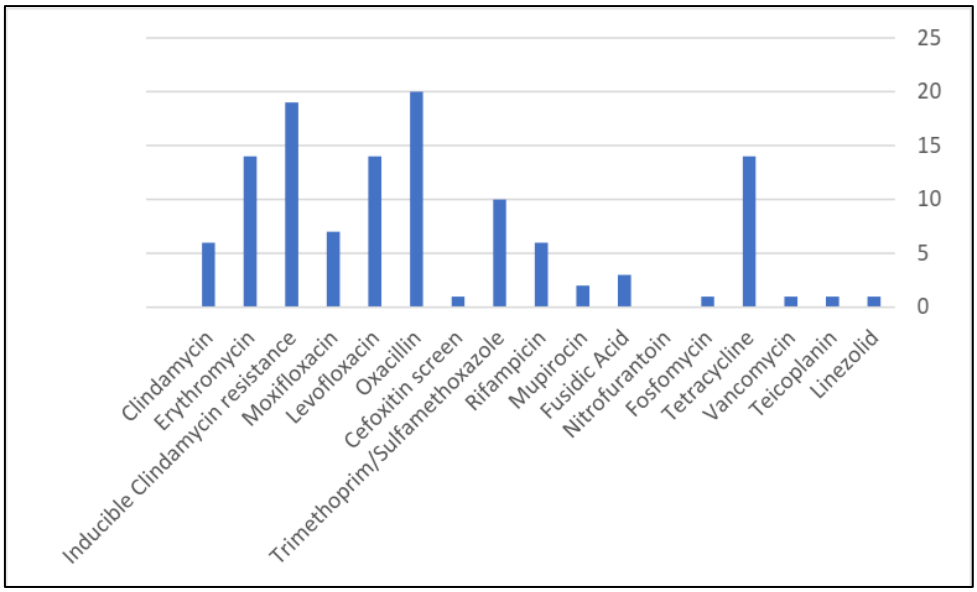

Fig 2: Results of applied 17 antibiotics against twenty-one bacterial isolates.

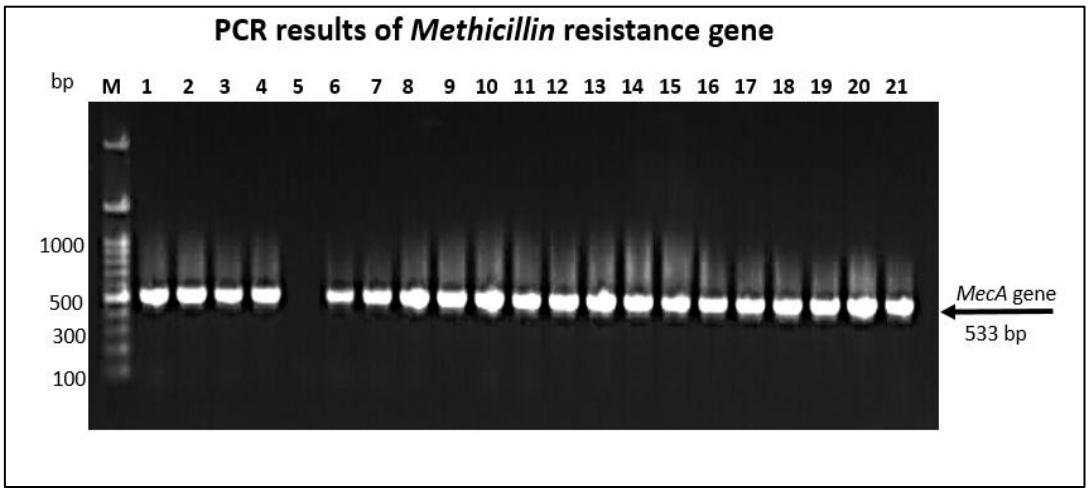

Figure 3: mecA gene amplification through PCR reaction with 533bps using specific forward and Reward primers.

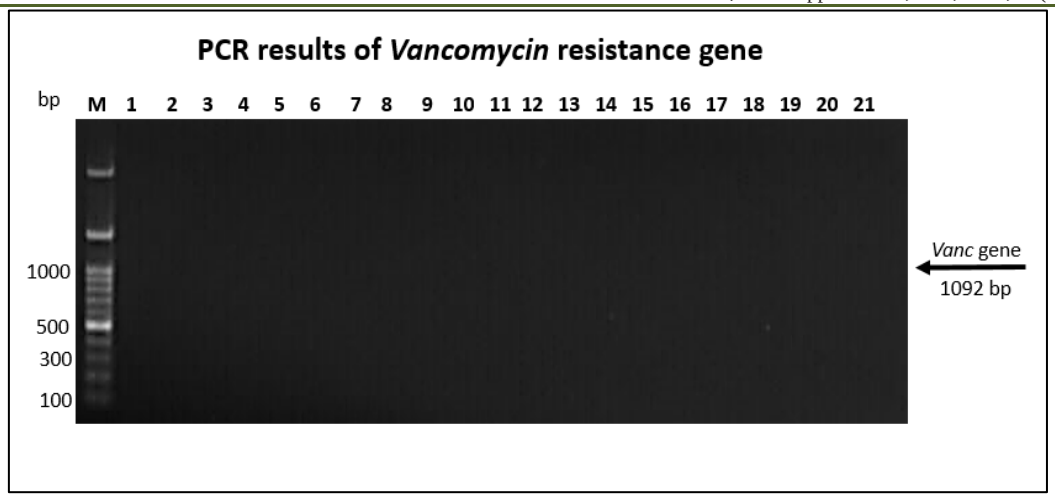

Figure 4: Vanc gene amplification through PCR reaction using specific forward and Reward primers.

DISCUSSION

In our current study of the prevalence of UTI in Saudi patients due to bacterial infections, almost our findings came parallel to previous reports of (Flores-Mireles *et al*., 2015) who confirmed the incorporation of the groups of pathogens including gram-positive and gram-negative bacteria as bacterial isolates in urinary tract infection in both males and females. Among our isolates, twenty-one were gram-positive, blood hemolytic isolates while others were gram- negative, which agreed with (Mohammed *et al*., 2016)(Guermazi-Toumi *et al*., 2018)(Salim, F. A; Murad, S. K; Elbareg, 2017)(Mostafa, M. M, Albakosh, A. M; Alrtail, A; Rzeg, M. M and Aboukay, 2016)Those who reported that the gram- negative bacteria were the most isolated bacteria from UTI. In our study, the mecA genes responsible for methicillin resistance were detected in Staphylococcus sp. using PCR, which was confirmed by another study in Saudi Arabia (Anwar *et al*., 2020). Who investigated 46 samples for methicillin resistance staphylococcus aureus (MRSA) detection and predicted that 45.8% were MRSA. Our finding detected high resistance among clinical isolates of S.haemolyticus. Our isolates of the mecA gene were susceptible to vancomycin this is consistence with previous research demonstrating that all mecA resistance strains are susceptible to vancomycin(Bathavatchalam *et al*., 2021). In conclusion, there are high mecA genes among clinical isolates in our study further studies with a high sample size of S.haemolyticous is recommended.

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COMPETING INTEREST: Not discloses

AUTHORS PARTICIPATIONS

K. A, A.dablool contributed to conceptualization, and analysis, M.sahran K.A (performed DNA extraction and DNA amplification)**.** Abdulmoghni E.A, Saad. A, Fayez. B (data curation, data collection data analysis) and all authors contributed to manuscript and final draft writing.

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