fortune Journals

Research Article

FORTUNE JOURNAL OF HEALTH SCIENCES

ISSN: 2644-2906

Retrospective Study of *Staphylococcus haemolyticus* in Blood Sample and their Susceptibility Pattern in A Tertiary Care Hospital in Dhaka, Bangladesh

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Abstract

Staphylococcus haemolyticus is one of the frequently isolated coagulase-negative Staphylococci(CoNS). Though it is considered mostly a skin contaminant, this organism has emerged as an important cause of nosocomial bloodstream infections (BSIs). A high antibiotic resistance profile and biofilm formation of *Staphylococcus haemolyticus* in comparison to other CoNS is a concern and therapeutic challenge now a days.

Aim: This study aims to observe the distribution of clinically significant *Staphylococcus haemolyticus* associated with bloodstream infection and to determine their antibiotic susceptibility profile.

Materials and Methods: A retrospective observational study which was conducted in the Department of Microbiology & Immunology, Bangabandhu Sheikh Mujib Medical University, Dhaka, Bangladesh, between June 2023 and July 2024. Six thousand, one hundred ninety-nine blood samples were collected in automated blood culture bottle, and the bacterial profile was retrieved using an automated BACT/ALERT 3 D System and BD BACTEC FX continuous monitoring system. The collected blood sample was processed, and the full identification of the organism and antimicrobial susceptibility was conducted by using the VITEK 2 Compact Lab automated system and Kirby Bauer disk diffusion methods per the National Committee for Clinical Laboratory Standards guidelines. Patients with suspected bacteremia with the clinical evidence of infection were included.

Results: A total of 6199 blood samples were collected, of which 519 (8.37%) showed bacterial growth. Among them, number of total isolated CoNS were 52(10.01%). The most prevailing isolate was *Staphylococcus haemolyticus* (59.61%), followed by *Staphylococcus hominis* (32.69%), *Staphylococcus urealyticus* (3.84%), *Staphylococcus epidermidis* (1.92%) and *Staphylococcus saprophyticus* (1.92%). High level of resistance was observed to ciprofloxacin (80.65%), followed by erythromycin (67.74%) and cloxacillin (51.61%). None of the isolates exhibited resistance to reserve drugs like vancomycin, linezolid and rifampicin. These isolates showed better susceptibility to amoxicillin (77.42%), cotrimoxazole (74.19%), gentamicin (67.7%). About 9.68% *Staphylococcus haemolyticus* was detected as methicillin resistant (MRSH) and they were sensitive to vancomycin, linezolid and rifampicin.

Conclusion: This result indicates that the isolated *Staphylococcus haemolyticus* among CoNS, is increasing, and an update of this isolate's antibiotic-resistance pattern is necessary for timely interventions and better patient outcome- as most of the isolates are considered as contaminant or remain underdiagnosed. These results underscore the implementation of effective infection prevention and control program in hospitals across Bangladesh.

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Citation: Rehana Razzak Khan, Ismet Nigar, SM Ali Ahmed, Sourav Debnath, Sharmeen Ahmed, Chandan Kumar Roy, Abu Naser Ibne Sattar, Sanjida Khandakar Setu, Shaheda Anwar. Retrospective Study of Staphylococcus haemolyticus in Blood Sample and their Susceptibility Pattern in A Tertiary Care Hospital in Dhaka, Bangladesh. Fortune Journal of Health Sciences. 7 (2024): 779-785

Received: December 16, 2024
Accepted: December 21, 2024
Published: December 30, 2024

Rehana Razzak K, et al., Fortune J Health Sci 2024
DOI:10.26502/fjhs.248

Keywords: *Staphylococcus haemolyticus*, VITEK 2 Compact, Bacterial pathogen, Antimicrobial resistance

Introduction

Coagulase-negative Staphylococci (CoNS) constitute the main microbiota of the skin. These pathogens were underestimated, and many microbiology laboratories did not include distinct species identification as they were considered as contaminant of skin [1]. About 40 different species of bacteria make up the heterogeneous CoNS group, and several of these have been identified as potential human pathogens [2]. *S. epidermidis, S. haemolyticus, S. saprophyticus*, and *S. hominis* are the most often isolated species from human specimens that cause disease [3,4]. *S. haemolyticus* is a part of skin microflora and one of the main species of CoNS [5]. These species accounts for 10–20% of clinical CoNS infections and is the second-highest species of CoNS in frequency and importance among isolates from clinical infections particularly from blood infections including sepsis [6,7]. It wasn't until the late 1960s that *Staphylococcus saprophyticus*, was linked to frequent urinary tract infections [8] In the following decades, the first cases of CNS infections in patients with invasive and indwelling medical devices were documented [9].

Several clinical infections are associated with *S. haemolyticus*, including bacteremia, meningitis, eye infections, skin infections, peritonitis, urinary tract infections, and male genital dysfunction [10,11]. *S. haemolyticus* is an emerging pathogen causing nosocomial infections [12]. The clinical relevance of CoNS-diagnosed by a single blood culture positivity is difficult to assess, however, some diagnostic reference standards for nosocomial BSIs are now available [13]. The clinical criteria essential for true bacteremia includes whether the patient has a fever or body temperature 38ºC and blood pressure ≤ 90mmHg. Also, other predisposing factors for such infection includes intravenous catheter or indwelling foreign devices, immunosuppressed patients, post-surgical infections, patients undergoing hemodialysis/ peritoneal dialysis, prolonged duration of hospitalization, and other laboratory infections [14,15]. *S. haemolyticus* is highly prevalent in hospitalized environments and tends to develop resistance to multiple antibiotics [16]. Many authors reported *S. haemolyticus* strains are resistant to one or more antibiotics amongst penicillin, cephalosporins, macrolides, tetracyclines, quinolones, aminoglycosides, glycopeptides [17,18,19]. Multi drug resistant *S. haemolyticus* strains spread in the hospital environment [20]. Without appropriate diagnosis and management of infections caused by *S. haemolyticus*, resistant strains of this pathogen can spread to other hospital settings and probably to the community [21]. This study aimed to identify *S. haemolyticus* and evaluate its resistance to commonly used antibiotics in patients with bloodstream infections as this organism has remarkable adaptability and survival capabilities within hospital environments, particularly on medical devices.

Materials and Methods

Study design

A retrospective study was done in the Department of Microbiology and Immunology at Bangabandhu Sheikh Mujib Medical University (BSMMU), Dhaka, Bangladesh from June 2023 to July 2024.

Study samples

Blood culture reports of patients presenting with symptoms of BSI were retrieved and analyzed. All the samples were collected from outpatients and admitted inpatients of BSMMU, who had clinical evidence of bloodstream infection. A total of 6199 automated blood samples were collected during that period. A comprehensive data regarding demographic data, previous antibiotic therapy, and laboratory results of bacterial isolation and susceptibility patterns were collected from the Laboratory specimen logbooks using the standard data collection form. The clinical criteria were determined as the presence of one or more of the following clinical factors based on the patient's history: fever >38.0°C or hypothermia 90 beats/minute, tachypnea >20 breaths/ minute, leukocytosis >12x10^9/l or leucopenia [14,22].

Laboratory Procedures

Sample collection

Adult and pediatric BACT/ALERT blood culture bottles were used. About 8-10 ml blood/bottle for adults and 3-5 ml blood/bottle for pediatric patients was collected in the blood culture bottle, labeled properly, and transported to the Microbiology laboratory without delay for the bacteriological examination.

Organism isolation and antimicrobial susceptibility

All the samples were incubated using an automated BACT/ALERT 3 D System and BD BACTEC FX continuous monitoring system. When the system indicated growth, blood agar and MacConkey agar media were used as solid culture media to isolate the organism. Organisms were identified based on morphology, culture characteristics, and biochemical reactions according to standard microbiological techniques. Culture showing significant growth of organism were identified to species level and AST was performed by VITEK 2 (bioMérieux, Inc., Durham, NC) system [23].All the isolates were also tested for antimicrobial susceptibility on Muller Hinton Agar (HI Media, India) by Kirby Bauer disc diffusion method, according to the Clinical Laboratory Standard Institute (CLSI) guidelines [24]. For gram-positive bacteria, the following antibiotics were used:

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amoxicillin(10µg), ciprofloxacin (5µg), cefradine (30 µg), cloxacillin (5µg), erythromycin (15µg), trimethoprim-sulphamethoxazole (1.25/23.75µg), vancomycin(30µg), linezolid (30µg). All the antibiotic disks were commercially purchased from Biomaxima, Poland. *S. aureus* ATCC 25923 were included as quality control strains of antimicrobial susceptibility testing. For the automated antimicrobial susceptibility VITEK Gram positive identification and AST card was used according to the manufacture's instruction. The antibiogram was obtained by VITEK 2 MIC system of the following antibiotics amikacin, gentamicin, clindamycin, erythromycin, cotrimoxazole, levofloxacin, ciprofloxacin, tetracycline, penicillin, teicoplanin, vancomycin, rifampicin, cefoxitin and linezolid.

Data analysis

Data were cleaned manually, entered and analyzed by using SPSS version 24 software. The statistical analysis used in the study was descriptive and did categorical data analysis. Frequency and percentage were examined for categorical independent variables. Results were presented through tables.

Results

A total of 6199 Automated blood sample were collected, of which 519(8.37%) yielded bacterial growth (Table 1). Among them coagulase negative Staphylococcus were 52(10.01%).

Table 1: Frequency of Bacterial isolates in Blood sample (n=6199)

Microbial Culture	Frequency	Percentage (%)
Growth	519	8.37
No growth	5680	91.62
Total	6199	100

Among them coagulase negative Staphylococcus (CoNS) were 52(10.01%). The most common isolate was *Staphylococcus haemolyticus*, which accounted for 31(59.61%), followed by *Staphylococcus hominis* 17(32.69%). The least isolated CoNS were *Staphylococcus urealyticus* 2(3.89%), *Staphylococcus epidermidis* 1(1.92%), *Staphylococcus saprophyticus* 1(1.92%) respectively (Table 2).

Table 2: Distribution of Coagulase negative Staphylococcus (CoNS) isolated from blood samples (n=52)

Species	No. of isolate	Percentage (%)
Staphylococcus haemolyticus	31	59.61
Staphylococcus hominis	17	32.69
Staphylococcus urealyticus	2	3.89
Staphylococcus epidermidis	1	1.92
Staphylococcus saprophyticus	1	1.92

Out of 31(59.61%) *Staphylococcus haemolyticus*, the majority 11(35.48%) were in the age group of over 60 years, and male were more commonly affected than female (58.06% vs 41.93%) patients. About 35.48% of the patients were above 60 years age, followed by 1-20 years (25.8%), 40-60 years (22.6%), <1 year (9.67%) and 20-40 years (6.45%) (Table 3).

Table 3: Characteristics of the study population of blood sample positive Staphylococcus

Characteristics	Frequency	Percentage
Sex		
Male	18	58.06
Female	13	41.93
Age		
< 1 yr	3	9.67
1-20 yrs	8	25.80
20-40 yrs	2	6.45
40-60 yrs	7	22.58
➢ 60 yrs	11	35.48

The antibiotic resistance pattern of the 31 *Staphylococcus haemolyticus*, isolated from blood sample was shown (Table 4). MIC breakpoints and zone diameter in the disk diffusion method were used according to Clinical Laboratory Standard Institute CLSI guidelines. The isolated *Staphylococcus haemolyticus* showed higher resistance to ciprofloxacin (80.65%), erythromycin (67.74%), cloxacillin (51.61%), and moderate resistance against gentamicin (32.26%), cotrimoxazole (25.81%) and amoxicillin (22.58%) respectively. However, it exhibited least resistance to amikacin (6.45%) cefradine (3.23%) and ceftriaxone (3.23%) respectively. Methicillin resistant *Staphylococcus haemolyticus* (MRSH) were 9.68%. However, the MRSH were 100% sensitive to vancomycin, linezolid, and cefuroxime.

Table 4: Antimicrobial resistance profile of the isolated *Staphylococcus haemolyticus* (n=31)

Antibiotics	Resistant	Percentage of resistance (%)
Amoxicillin	7	22.58
Cloxacillin	16	51.61
Erythromycin	21	67.74
Cefradine	1	3.23
Trimethoprim-Sulfamethoxazole	8	25.81
Ciprofloxacin	25	80.65
Ceftriaxone	1	3.23
Gentamicin	10	32.26
Amikacin	2	6.45
Cefoxitin	3	9.68

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Discussion

Staphylococcus haemolyticus, among CoNS, constitutes the main part of human skin microbiota. It is widespread in hospitals and among medical staff resulting in nosocomial infection. The rate of bacterial isolation in this study was 8.37%, which is comparable with the study result conducted in India (14%) but in Ethiopia the isolation rate was higher (28%) [25-28]. This differences in the bacterial isolation rate might be due to sample collection method adopted and infection control practices among institutions. In the present study, after considering the clinical details and laboratory criteria, CoNS were considered true pathogens in bloodstream infections in 10.01% of cases. Bhosle et al. did a similar study in India and reported CoNS as a true pathogen in 15.6% of cases [29], and Sindhu et al. reported 24% in Amritsar [14]. The most frequently isolated CoNS species in the present study were *Staphylococcus haemolyticus* (59.61%), followed by *Staphylococcus hominins* (32.69%), and *Staphylococcus urealyticus* (3.89%). Verma et al., had reported *Staphylococcus haemolyticus* as the commonest CoNS spp. (52.9%) causing bloodstream infection followed by *S. hominis* (29.4%) and *S. epidermidis* (15.3%) [30]. The higher number of *S. haemolyticus* isolates in our study might be attributed to the inclusion of hospitalized patients and different procedures specially device associated, undertaken after hospitalization increases the risk of acquiring infection by this pathogen. This differentiation between skin contaminant, and pathogenic strain also largely depends on the virulence strategies employed by the various species, as well as the host's defense mechanisms [31].

However, this finding is not in accordance with other studies, where *Staphylococcus epidermidis* constituted the predominant species, (50.8%) [31,32,33]. These studies had reported *Staphylococcus haemolyticus* as the second most frequently isolated CoNS strain from blood cultures. It is also the second most frequent cause of nosocomial infections in patients on medical device [33,34]. The majority of *S. haemolyticus* isolates in our study were from the age group above 60 years, and males were predominant, and the finding was consistent with Bhosle. et al. [29]. Prolonged hospitalization might be the one of the probable causes of this age group. They are most found in elderly patients or individuals who are immunosuppressed, and these patients tend to be broadly susceptible to antibiotic treatments [35]. However, Verma et al. reported an age group of 18-60 years, with male predominance in their study [30]. To our knowledge, this age and sex relationship of *S. haemolyticus* requires further evaluation.

In our study, the antibiotic susceptibility patterns showed that *S haemolyticus* was highly resistant to ciprofloxacin, erythromycin, and cloxacillin at 80.65%, 67.74%, and 51.61%, respectively. This correlated with a study done by Rania et al., where *S. haemolyticus* among CoNS showed higher resistance to ciprofloxacin (74.3%) [36]. Widespread and non-judicious use of antibiotics, self-medication, low cost, and availability of antibiotics in developing countries like Bangladesh might promote the development of resistance to these antibiotics. However, other studies reported lower resistance to ciprofloxacin (64-66.7%), and but higher resistance to erythromycin, (73.3 to 77.8%) [37,38].

In our study, resistance to amoxicillin was 22.58%; however, many studies reported high resistance to amoxicillin (80% to 88.9%) [39,40]. In the present study, cefradine and ceftriaxone showed lower resistance, 3.23% and 3.23%, respectively. This higher sensitivity to beta-lactam and cephalosporin in our study might be due to the less common practice of this drug in our patients. None of the isolates in the present study showed resistance to vancomycin, rifampicin and linezolid, like previous studies [41]. A recent study by Barros et al. (2012) found that 75% of the *S. haemolyticus* isolates examined exhibited multiresistance [42]. This species also plays a significant role in the spread of resistance genes, facilitating the emergence of epidemic clones of the more virulent nosocomial pathogen, *S. aureus*. Another two studies showed 15% resistance to vancomycin [41,43]. Factors like indiscriminate use of glycopeptides and beta-lactam and frequent hospitalizations act as important risk factors for developing resistance to glycopeptides [40]. The first cases of *S.haemolyticus* strains resistant to linezolid have been reported in India and various European countries [44,45].

Methicillin-resistant *Staphylococcus haemolyticus* MRSH was 9.68% in the present study, much lower than other studies (75%- 88%,) [41,44]. However, this varied geographical distribution of strains, as well as discordant findings between phenotype test and genotypic characterization of various strains of *S. haemolyticus* [41,45]. These multidrug-resistant, skin-colonizing bacteria not only pose a risk for the emergence and spread of nosocomial infections but can also infect healthcare personnel and visitors to patients [46]. Additionally, *S. haemolyticus* has demonstrated resistance to disinfection. Molecular typing of infections caused by MRSH, collected over a three-year period from a neonatal ICU, revealed that *S. haemolyticus* can survive in disinfectant solutions. This survival ability enables the bacteria to serve as a reservoir, potentially infecting newborns.

Conclusion

To our knowledge, this is the first evaluation of *Staphylococcus haemolyticus* among CoNS-causing BSI from Bangladesh. Clinicians and microbiologists often face the challenge of determining whether isolated CoNS are contaminants introduced during sampling or processing, harmless commensals of the skin or mucous membranes,

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DOI:10.26502/fjhs.248

or clinically relevant pathogens. This species is now a worldwide concern for its drug resistance. Proper blood collection techniques, training and practice of hand hygiene, infection control policy, and implementation of the antibiotic restriction policy can help to prevent hospital-acquired infection by this pathogen.

Limitation

Due to the study design, we were unable to collect two consecutive samples of all the patients. So, laboratory correlation with the clinical data was limited to some extent.

Acknowledgement

We thank the entire technical team of the Microbiology Laboratory, Microbiology & Immunology Department, Bangabandhu Sheikh Mujib Medical University.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

Authors Contributions

All authors contributed equally to this work.

Funding

This study did not receive any funding.

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Citation: Rehana Razzak Khan, Ismet Nigar, SM Ali Ahmed, Sourav Debnath, Sharmeen Ahmed, Chandan Kumar Roy, Abu Naser Ibne Sattar, Sanjida Khandakar Setu, Shaheda Anwar. Retrospective Study of Staphylococcus haemolyticus in Blood Sample and their Susceptibility Pattern in A Tertiary Care Hospital in Dhaka, Bangladesh. Fortune Journal of Health Sciences. 7 (2024): 779-785