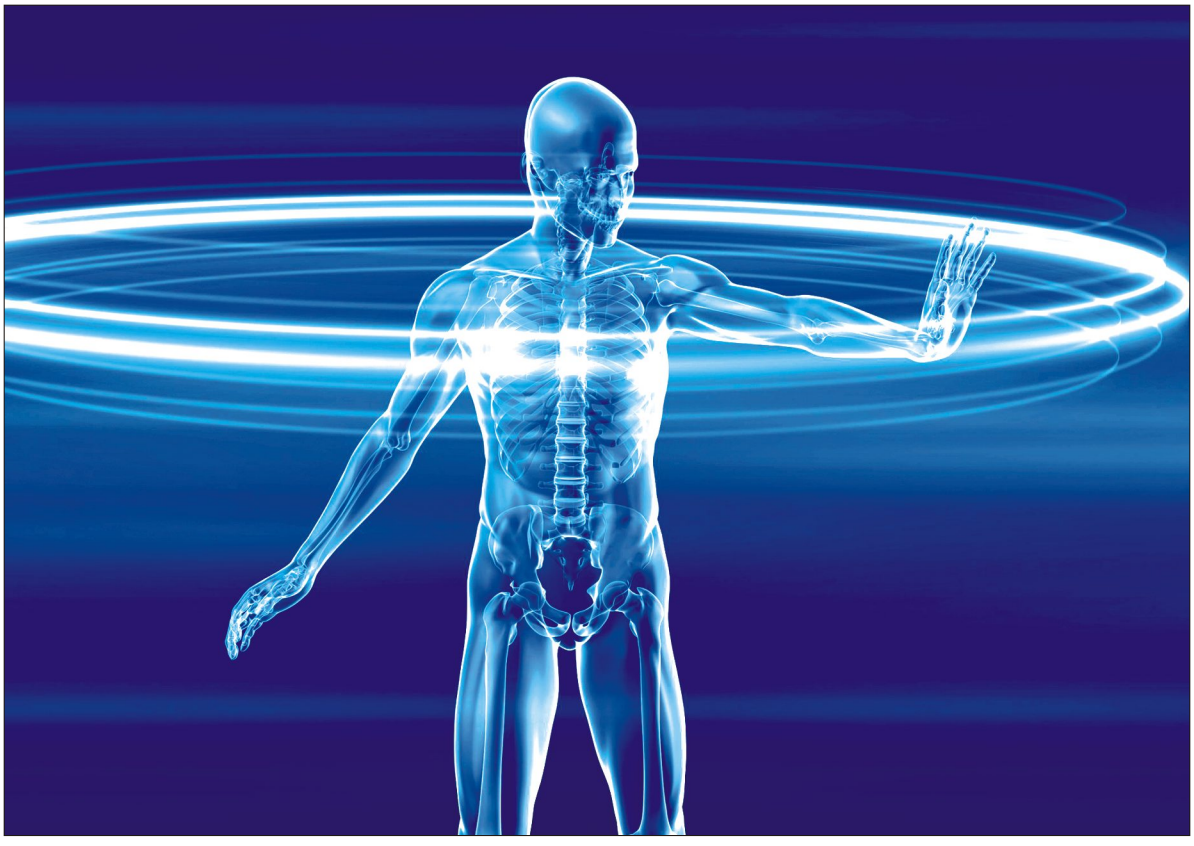

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Research Article
Spectrum of Pathogens Isolated from Patients with Hemolytic Diseases

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Abstract

Background and Objectives: Patients with hemolytic diseases are at higher risk of infections. Moreover, infections are significant contributors to morbidity and mortality in patients with hemolytic disease. Frequent screenings of infections, the spectrum of the organism, site of infection like blood urine sputum. Details of bacteremia must be explored and treated earliest to reduce morbidity and mortality in patients with hemolytic disease. The study has aimed to identify the prevalence of infection in patients with hemolytic disease in the Saudi population. **Materials and Methods:** A total of 113 samples were examined for microbial growth on six different culture media (BAP, NA, MacConkey, CAP, *Salmonella* Agar and PDA), At microbiology and toxicology laboratories at in Security Forces Hospital and King Fahd Medical City in Riyadh Saudi Arabia. **Results:** The characteristics of 113 episodes of invasive bacterial infection among patients with hemolytic diseases were examined. Salient pathogens were *K. pneumoniae* MDR, tissue MARSA, AFB tissue, *E. coli*, *Pseudomonas aeruginosa*, *H. pylori*, *C. arbiran*, *Enterobacter cloacae*, HBS Group A, H1N1, *Candida albicans*. The most frequent pathogen was *K. pneumoniae* MDR, tissue MARSA, the most frequent pathogen in the urine was *K. pneumoniae* and *E. coli*. Pathogen isolated in the sputum were *Pseudomonas aeruginosa*, *K. pneumoniae*, *Acinetobacter*. The most frequent pathogen in the stool was *Clostridium* Bacteremia was caused by tissue MARSA, *S. aureus*, *Enterobacter*, *K. pneumoniae*. **Conclusion:** Infection is prevalent in patients with hemolytic diseases. *K. pneumoniae* tissue MARSA, AFB, *E. coli* 9 (7.96%) and *Pseudomonas* were salient pathogens. Patients with hemolytic diseases should be investigated early and aggressively for infection.

Key words: Infections, hemolytic disease, *K. pneumoniae*, bacterial, spectrum, pathogens, tissue MARSA

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Infections in patients with Hemolytic diseases are one of the major health concerns globally. Worldwide, millions of people suffer from hemolysis, the breakdown of red blood cells, such as those troubled with sickle-cell disease, thalassemia, malaria or sepsis. These patients face an exceptional risk of death from bacterial infections.

Patients with hemolytic diseases are at higher risk of infections. Also, hemolysis predisposes to infection. The mortality, due to septic shock as a result of blood infection, is one of the common observations in the cases of Hemolytic disease thalassemia[1].

Patients with hemolytic disease require a regular blood transfusion for their management. It exposes them to bacterial infection and produces excess iron in patients' bodies and also provides a suitable environment for bacteria[2].

Infections are significant contributors to morbidity and mortality in patients with hemolytic disease. Further Infection precipitate crisis in sickle-cell disease. Surveillance of infection is necessary for reducing the global burden of mortality in sickle-cell disease[3].

The infection has remained an important cause of death in patients with hemolytic disease[4-6]. A deep association between hemolysis and bacterial co-infection in hemolysis is documented in the literature[7].

Infections cause hemolysis by producing a hemolysin (e.g., *Clostridium perfringens*), by stimulating an immune response (e.g., *Mycoplasma pneumoniae*), infiltration of Red Blood Cells (RBCs) or by oxidative damage to RBCs[8].

Massive intravascular hemolysis is a known complication of *Clostridium perfringens* bacteremia. Intra vascular hemolysis and a very high mortality rate occur due to the toxin of *Clostridium perfringens*[9,10]. Although infections are a risk factor for morbidity, hospital admissions, prolonged hospital stay and mortality. Yet, infections are an underestimated and under-investigated issue in patients with hemolytic disease.

Hemolytic diseases are relatively frequent in Saudi Arabia. And sickle cell disease is a common genetic disorder of variable prevalence in different regions of Saudi Arabia[11,12].

SCD is prevalent in Saudi Arabia. Children with sickle cell disease are at increased risk of developing bacteremia and other serious bacterial infections[13].

Overwhelming infections are an important cause of morbidity and death in patients with sickle cell disease. Usual pathogens are *S. pneumoniae* and *H. influenzae* type b and non-typhoid salmonellosis. Seventy percent of septicemias and meningitis with sickle cell disease is caused by *S. pneumoniae*[3].

Frequent screenings of complete spectrum of infectious complications like; type of organism involved, bacteria, fungus or virus infection; site of infection like blood urine sputum or other. Details of bacteremia must be explored to plan preventive and prophylactic strategies for these patients.

This study was conducted to explore the prevalence and type of infection-causing pathogens in hemolytic patients.

MATERIALS AND METHODS

Study area: The study was conducted in the Department of Biology and Security Forces Hospital, Riyadh, Saudi Arabia from October, 2018-August, 2019.

Sample collection: A total of 113 isolates were collected for microbiological analysis. Analysis was performed in the microbiology and toxicology laboratories at in Security Forces Hospital and King Fahd Medical City in Riyadh, Saudi Arabia.

Methodology: Samples were explored for the bacteria, virus or fungal infections, on different culture media. Salient media were Agar plate, Nutrient Agar, MacConkey Agar, BAP, CAP, Salmonella Agar and PDA for pathogen growth. One millilitre of each specimen was diluted in 99 cm^3 of sterilized distilled water and 1 mL of the mixture was incubated at 30°C for 48 hrs. Three replications of each specimen were prepared.

Microorganism identification: Identification of microorganisms was done using the API 20E system (Analytical Profile Index, Biomerieux, Durham, NC, USA. https://www.biomerieux-usa.com/sites/subsidiary_us/files/18_api-ref-guide_v7.pdf.).

RESULTS

The spectrum of pathogens in the isolates is shown in Table 1. The prominent pathogens are *Klebsiella pneumoniae* MDR 13 (11.5%), tissue MARSA 13 (11.5%), AFB tissue 12 (10.61%), *E. coli* 9 (7.96%), *Pseudomonas aeruginosa* 9 (7.96%), *H. pylori* 3 (2.65%), *C. arbiran* 3 (2.65%), *Enterobacter cloacae* 2 (1.76%), HBS Group A were 14 (12.38%), H1N1 7 (6.2%), *Candida* white was 6 (5.3%),

The data in Table 2 also displays the pathogens isolated from inpatients, the most common pathogen was *Staphylococcus aureus* 1 (0.88%), *Clostridium* 4 (3.53%), *E. coli* 4MDR, *Pseudomonas aeruginosa* 8(7.07%), *K. pneumoniae* MDR 6 (5.3%), AFB tissue 10 (8.84%), tissue MARSA 9 (7.96%), *Candida albicans* 6 (5.3%), *Acineto baumannii* 1 (0.88%), *Enterococcus faecium* va I (MDR) 1 (0.88%), *H. pylori* 3 (2.65%), *Acinetobacter*, *C. arbican* 3

Table 1: Frequency of salient pathogens in the overall isolates from patients with hemolytic disease

Pathogens	Frequency (%)
Staphylococcus aureus	2 (1.76)
Clostridium	5 (4.42)
Escherichia coli	9 (7.96)
Pseudomonas aeruginosa	9 (7.96)
Klebsiella pneumoniae MDR	13 (11.5)
AFB tissue	12 (10.61)
Tissue MARSA	13 (11.5)
Candida albicans	6 (5.3)
Acineto baumannii	1 (0.88)
Enterococcus faecium va I (MDR)	1 (0.88)
Helicobacter pylori	3 (2.65)
Acinetobacter	1 (0.88)
C. arbiran	3 (2.65)
C. arbican	2 (1.76)
Enterobacter cloacae	2 (1.76)
Salmonella	1 (0.88)
Diphtheroids pnob	1 (0.88)
Viridans stnopt Rrectal	1 (0.88)
Yeast	1 (0.88)
HVS	2 (1.76)
H1N1	7 (6.2)
HBS group A	14 (12.38)
HBS group B	
Corona	8 (7.07)

N = 113

Table 2: Frequency of pathogens in the isolates from patients with hemolytic disease

Pathogens	Other (%)
Staphylococcus aureus	1 (0.88)
Clostridium	4 (3.53)
Escherichia coli	4MDR
Pseudomonas aeruginosa	8 (7.07)
Klebsiella pneumoniae MDR	6 (5.3)
AFB tissue	10 (8.84)
Tissue MARSA	9 (7.96)
Candida albicans	6 (5.3)
Acineto baumannii	1 (0.88)
Enterococcus faecium va I (MDR)	1 (0.88)
Helicobacter pylori	3 (2.65)
Acinetobacter	
C. arbican	3 (2.65)
Enterobacter cloacae	1 (0.88)
Enterobacter cloacae	2 (1.76)
Salmonella	
Diphtheroids pnob	1 (0.88)
Yeast	1 (0.88)
HVS	2 (1.76)
H1N1	7 (6.2)
HBS group A	14 (12.4)
HBS group B	
Corona	8 (7.07)
Other *Ralstonia pickettii*	
Burkholderia cepacia, Trach	7 (6.2)

N = 113

(2.65%), *Enterobacter cloacae* 1 (0.88%), *Enterobacter cloacae* 2 (1.76%), *Salmonella*, *Diphtheroids pnob* 1 (0.88%), Yeast 1 (0.88%), HVS 2 (1.76%), H1N17 (6.2%), HBS Group A 14 (12.4%), HBS Group B, Corona 8 (7.07%), Other *Ralstonia pickettii*, *Burkholderia cepacia*, Trach 7 (6.2%).

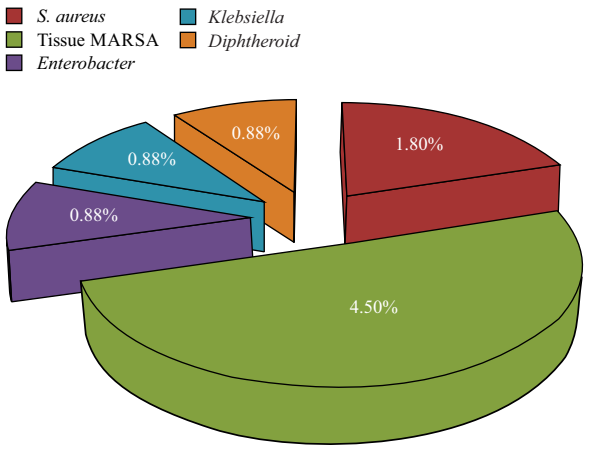

Fig. 1: Bacteraemia occurred by tissue MARSA *S. aureus*, *Enterobacter, Klebsiella* and *Diphtheroid*

Table 3: Frequency of pathogens in the isolates from patients with hemolytic disease

Pathogens	Urine	Sputum	Stool
Escherichia coli	5 (4.42%)		
Pseudomonas aeruginosa		1 (0.88%)	
Klebsiella pneumoniae MDR	5 (4.42%)	1 (0.88%)	
AFB tissue		1 (0.88%)	
Tissue MRSA		1 (0.88%)	
Clostridium			7 (6.2%)
	Ova rota		3 (2.65%)
Acinetobacter		1 (0.88%)	
Enterobacter cloacae	1 (0.88%)		

N = 113

The virus was H1N1 7 (6.2%), HVS 2 (1.76%) HBS Group A 14 (12.4%), Corona 8 (7.07%).

The data in Table 3 shows the most common bacteria in saliva, urine and stool. The most common pathogen in urine was 5 (4.42%), followed by the frequent *K. pneumoniae* at least bacteria was *Enterobacter cloacae* 1 (0.88%). The pathogen isolated in sputum is *P. aeruginosa* 1 (0.88%), *K. pneumoniae* 1 (0.88%), *Acinetobacter* 1 (0.88%). The most common pathogen in stool was *Clostridium* 7 (6.2%), tissue bacteremia MARSA (0.88%), *S. aureus* 1.8%, *Enterobacter* 1 (0.88%), *K. pneumoniae* 1 (0.88%). The data in Fig. 1 Spores are caused by tissues of MARSA (4.50%), *S. aureus* (1.80%), *Enterobacter* (0.88%), *Klebsiella* (0.88%) and *Diphtheroid* (0.88%).

DISCUSSION

Patients with hemolytic disease represent a special subgroup of patients who are particularly more prone to infections and bacteremia. It is worth noting that these patients should be thoroughly screened and investigated for the possible reasons for increased susceptibility for infections,

to improve the outcome. Although being risk factors for mortality, infections are an underestimated issue in patients with hemolytic disease. It is mandatory to reduce mortality by recognizing and presumptively treating infections in these patients as quickly as possible. The incidence of bacterial infection is higher in children with the hemolytic disease globally 16% compared to 3-14% in general children. It is responsible for 12-46% of patient deaths[14].

In the present study, *Klebsiella pneumoniae* 13 (11.5%) and tissue MARSA 13 (11.5%) were the most common organism followed by *Pseudomonas* 9 (7.96%) and *E. coli* 9 (7.96%). In other studies, worldwide, *Klebsiella* species were responsible for 25% of all severe infections in patients with thalassemia in Thailand. *Escherichia coli* (26% of infections), *Salmonella* species (15% of infections) and *Streptococcus pneumoniae* (13% of infections)[4-15].

In the present study, Bacteraemia occurred by tissue MARSA *S. aureus*, *Enterobacter*, *Klebsiella* and *Diphtheroid*. Massive intravascular hemolysis is a known complication of *Clostridium perfringens* bacteremia. Intravascular hemolysis and a very high mortality rate occur due to the toxin of *Clostridium perfringens*[10]. Hemolytic diseases are relatively frequent in Saudi Arabia. And sickle cell disease is a common genetic disorder of variable prevalence in different regions of Saudi Arabia.

Infections are a more common cause of hospitalization and are considered to be a life-threatening condition[11,16]. Patients with hemolytic disease require a regular blood transfusion for their management. Making them vulnerable to acquiring infections. They also develop iron overload in their body, which may provide a likely environment for the bacteria to flourish[2]. *Klebsiella pneumoniae* 13 (11.5%) was the most common organism in this study. In a study by Joy *et al.*[17], the incidence of pneumococcal septicemia was found to be 2.5 episodes per 100 patients in years.

Given the poor outcome of infection, prompt treatment with antibiotics and early surgical intervention if needed should be initiated. Whenever there is suspicion of *Klebsiella* infection. Future studies should be performed to address predisposing factors.

CONCLUSION

In the present study infections (bacterial viral and fungal) were frequent in patients with hemolytic disease. *Klebsiella* and MRSA infection was found to be an important complication of Hemolytic disease in KSA and were involved in causing bacteremia. Knowing the poor outcome of infection, clinicians should be aware of any potential infections when taking care of patients with hemolytic disease. Prompt treatment including a reasonable choice of antibiotics should be initiated, whenever, there is suspicion of MARSA or *Klebsiella* infection. Future studies should be performed to address predisposing factors for infections in patients with hemolytic disease. Frequent surveillance for pathogens is desired as there is a greater risk of bacteremia in these patients. They need aggressive investigation and management.

SIGNIFICANCE STATEMENT

This study revealed the infectious diseases that can affect hemolytic patients and that can lead to an exacerbation of their infection in the absence of early therapeutic intervention. The importance of the study lies in revealing the types of bacteria, viruses and fungi that may infect the patient, which facilitates the selection of the appropriate protocol to eliminate the infection. In addition to the preventive measures that can be taken in this aspect.

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