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Changing Trends in Invasive Streptococcal Infections: Experience in a Tertiary Care Hospital, Saudi Arabia

Elham E. Bukhari[1*], Fahad Almogilaith[2], Fawzia Alotaibi[3], Akram M Nurhussen[4]
[1]Department of Pediatric, Pediatric Infectious Disease unit , College of Medicine, King Saud University and King Saud University Medical City, Riyadh, Saudi Arabia
[2]Department of Pediatric, Infectious Disease, King Saud Medical Complex Riyadh Riyadh, Saudi Arabia
[3]Department of Pathology, College of Medicine, King Saud University and King Saud University Medical City, Riyadh, Saudi Arabia
[4]Department of Pediatric, College of Medicine, King Saud University and King Saud University Medical City, Riyadh, Saudi Arabia

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***Corresponding author**
Fawzia E. Alotaibi

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Abstract: Streptococci cause a diverse array of infections. Changing trends in Streptococci causing invasive disease was noticed. This analysis aims to elucidate epidemiological trends amongst various invasive streptococcal diseases (IPD). Surveillance data was abstracted from the Electronic microbiology System for confirmed cases of alpha and Beta-hemolytic streptococci. A total of 91 isolates of streptococci were included. Streptococcus mitis 23 (23.9 %), was the most common isolate followed by Streptococcus agalactiae (Group B) 20 (20.8%) Streptococcus pneumoniae 14 (14.5%) Streptococcus pyogenes (Group A) 7 (7.29%), Streptococcus salivarius 7 (7.29%) and 20other different streptococcal species including (Streptococcus gordonii 5 (5.2%), Streptococcus parasanguinis5 (5.2%), Streptococcus sanguinis 4 (4.1%) Streptococcus species 1 (1%) Streptococcus anginosus 1 (1%), Streptococcus gallolyticus 1(1%), Streptococcus infantarius 1 (1%), Streptococcus intermedius 1 (1%), Streptococcus thermophilus 1 (1%)). Of 91 isolates identified cases of invasive disease, Children ≥1-5 years had the highest incidence for Invasive streptococcal disease (20.4%), Among the IPD, Bacteremia was the most frequently reported clinical manifestation for 64 (70.3%), followed by Invasive soft tissue infection 18 (19.7%) then meningitis 9 (9.8%). There is high prevalence of invasive disease in the study population.

Keywords: Streptococci, infections, invasive disease, microbiology, Streptococcus mitis.

INTRODUCTION

Streptococci are classified as Gram-positive cocci based on their appearance under a microscope. Streptococci are further classified into subtypes based on sugar chains expressed on their outer shell (Lancefield group) and their behavior when grown in the laboratory (alpha- or beta- haemolysis). Most beta hemolytic streptococci (BHS) belong to the Lancefield groups A, C and G. Beta-hemolytic streptococci (βHS) are known to cause a diverse set of clinical presentations for example Group A streptococci (GAS) constitute the most important and prevalent members of this group(BHS) causing clinical syndromes ranging from self-limited pharyngitis and impetigo to potentially life-threatening toxic shock syndrome. Alpha-haemolytic made up of two groups, Streptococcus pneumoniae and Viridans group streptococci (VGS).Streptococci pneumoniae (pneumococci) are bacteria important in pneumonia and meningitis but rarely cause skin disease [1]. Viridans group streptococci (VGS), a genetically heterogeneous group of bacteria, are the predominant bacteria in the human oropharynx, VGS include: mitis, oralis, infantis /australis, Sanguinis, Anginosus, Salivarius /Vestibulari. VGS cause a wide range of infections in humans, including bacteremia in patients with neutropenia, infective endocarditis, and orbital cellulitis [2–5].

Streptococcal Invasive disease can have complex manifestations that require intensive clinical management, such as meningitis and sepsis. Few studies have investigated invasive diseases collectively. This analysis aims to elucidate epidemiological trends and clinical presentation amongst various Streptococcal invasive diseases in Saudi Arabia.

METHOD

The study was conducted at King Saud medical city, an 11,00 bed tertiary care hospital in Saudi Arabia. The Streptococcal isolates included in this study

were recovered from clinical samples from all different medical service of the hospital including intensive care units. All the BHS and alpha-hemolytic (n = 91) data was recovered from Electronic microbiology System over a period between February 2015 and March 2017. The isolates were collected from various clinical samples of patients admitted to the hospital. A detailed clinical history was recorded for all the patients. All the isolates of βHS were identified by standard microbiological methods The confirmation of identity was done using the Vitek 2 identification cards (bioMérieux, Marcy l'Etoile, France) [6]. Grouping of the streptococci was performed using an agglutination test (HiMedia Labs, Mumbai, India) according to the manufacturer's instructions. All the strains were stocked in stocking beads (Microbank, Pro-Lab Diagnostics, Richmond Hill, Canada) at 70°C until further analysis [7]. Viridans group streptococci (VGS): Bacterial isolates were identified as VGS on the basis of the following: presence of α-hemolysis, gram-positive reaction, coccus morphology arranged in chains, negative catalase test results, and exclusions of pneumococcus and enterococci by routine biochemical tests (i.e., optochin, bile solubility, and pyrrolidonyl arylamidase tests). VGS species was determined as described.. [8]. *Streptococcus pneumoniae* isolates were cultured on 5% sheep blood Columbia agar (bioMérieux) for 18 h to 48 h at 35°C under a 5% CO_2 atmosphere. A 5-µg optochin disk (6-mm disks; MAST ID Optochin Discs, MAST, United Kingdom) was placed on sheep blood Columbia agar plates inoculated under 5% CO_2 atmosphere, or ambient atmosphere when stated. Optochin susceptibility was defined as an inhibition zone of ≥14 mm. Bile solubility test [9]. The bile solubility test was performed with the tube method, with preparation of bacterial suspensions in 1 ml of 0.9% NaCl equivalent to a McFarland 1.0 standard. A 0.5-ml portion of 2% deoxycholate was added to a 0.5-ml suspension of each isolate prepared in 0.9% NaCl and incubated at 35°C for 1 h. A positive test was indicated by visible clearing of the suspension. A negative control for each isolate was similarly performed with 0.5 ml of 0.9% NaCl added to a 0.5 ml suspension of each isolate prepared in 0.9% NaCl. Pneumococcal capsular polysaccharide serotype was determined by agglutination with specific antisera (Statens Serum Institut, Copenhagen, Denmark) [10].

Antimicrobial susceptibility testing

The antimicrobial susceptibility testing of streptococci was performed using the disk diffusion method on Mueller Hinton agar with 5% sheep's blood according to the recommendations of the CLSI. [11,12]. The following antibiotics were tested: penicillin G, ampicillin vancomycin, erythromycin, clindamycin, cefotaxime, ceftriaxone. *S. pneumoniae* ATCC 49619 was used as a control. The minimum inhibitory concentration (MIC) was also determined by the E-test for all the above antimicrobials; the test was performed according to manufacturers.

RESULTS

A total of 91 isolates of streptococci were included. The Streptococcal isolates included in this study were recovered from clinical samples from different wards of the hospital. (Table1). All positive isolates were collected from clinical samples including blood, cerebrospinal fluids, soft tissue of patients admitted to the hospital. (Table 2). *Streptococcus mitis* 23 (23.9 %), was the most common isolate followed by *Streptococcus agalactiae* (Group B) 20 (20.8%) *Streptococcus pneumoniae* 14 (14.5%) *Streptococcus pyogenes* (Group A) 7 (7.29 %) *Streptococcus salivarius* 7 (7.29%), *Streptococcus gordonii* 5 (5.2%), *Streptococcus parasanguinis 5 (5.2%), Streptococcus sanguinis.* 4 (4.2%), *Streptococcus species 1 (1%), Streptococcus anginosus* 1 (1%), *Streptococcus gallolyticus* 1 (1%), *Streptococcus infantarius* 1 (1%), *Streptococcus intermedius* 1 (1%), *Streptococcus thermophilus* 1 (1%) (Table 3). Of 91 isolates identified cases of invasive disease, Age distribution of patients with invasive streptococcal infection was including children less than one year old to 70 years old .(figure.1). Children ≥1-5 years had the highest incidence for Invasive streptococcal disease (20.4%), followed by those more than 70 years (18.4%)). Among the IPD, Bacteremia was the most frequently reported clinical manifestation for 64 (70.3%), followed by soft tissue infection 18 (19.7%) then meningitis 9 (9.8%). Among the bacteremia episode there was 17 *Streptococcus mitis* isolated followed by 12 *Streptococcus pneumoniae, 12 Streptococcus agalactiae* (Group B), 5 *Streptococcus pyogenes* (Group A), 5 *Streptococcus salivarius,* and 13 collective of different streptococcal species. There was 9 cases of meningitis caused by *Streptococcus pneumoniae* isolated from 5 cases, *Streptococcus agalactiae (Group B)* isolated from 3 cases, *Streptococcus sanguinis* isolated from 1 case. For the invasive soft tissue infection there was 18 episode , the following streptococcus was isolated ; 4 *Streptococcus mitis*, 3 *Streptococcus sanguinis*, 3 *Streptococcus parasanguinis* 2 *Streptococcus agalactiae* (Group B), 2 *Streptococcus pyogenes* (Group A) , 2 *Streptococcus species*., 1 *Streptococcus gordonii*, 1 *Streptococcus intermedius.* The susceptibility pattern of the streptococcal isolates to the most commonly used systemic antibiotics like penicillin 59 (60.2%), ceftrixone was 86 (87.8%), and for vancomycin 97(99%). Underlying disease that involved different system like cardiovascular, central nervous system, nephrology system, endocrine system, hematology/oncology system and immune system associated with invasive streptococcal infection was also recorded. (Table. 4) There was no statistical difference between the underlying diseases and the invasive streptococcal infection.

Table-1: Site of admission of patients with Invasive Streptococcal Disease

Site of admission	Frequency	Percent
Coronary Care Unit	1	1%
Medical Clinic	5	5.1%
Emergency Room	5	5.1%
Medical Wards	26	26.5%
Neonatal Intensive Care Unit	2	2%
Obstetrics/Gynecology wards	7	7.1%
Oncology Ward	1	1%
Pediatric Wards	31	31.6%
Surgical Wards	13	13.4%
Total	91	100

Table-2: Percentage of positive Streptococcal isolates collected from clinical samples including blood, cerebrospinal fluids, soft tissue of patients admitted to the hospital

Specimen	Frequency	Percent
Body fluid	8	12 %
Blood	69	69 %
CSF	9	9 %
Tissue	6	6 %
Total	91	100

Table-3: Percentage of Different Streptococcal Species Involved In Invasive Streptococcal Infection

Species	Frequency	Percent
Streptococcus mitis	23	23.9%
Streptococcus agalactiae (Group B)	20	20.8%
Streptococcus pneumoniae	14	14.5%
Streptococcus pyogenes (Group A)	7	7.29%
Streptococcus salivarius	7	7.29%
Streptococcus gordonii	5	5.2%
Streptococcus parasanguinis	5	5.2%
Streptococcus sanguinis	4	4.2%
Streptococcus species		
Streptococcus anginosus	1	1%
Streptococcus gallolyticus	1	1%
Streptococcus infantarius	1	1%
Streptococcus intermedius	1	1%
Streptococcus mutans	1	1%
Streptococcus thermophilus	1	1%
Total	91	100

Fig-1: Age distribution of patients with invasive streptococcal infection (<1year->70year)

Table-4: Underlying disease associated with invasive streptococcal infection

Underlying diseases		
Cardiovascular disease		
Congenital Heart Disease	1	1
Heart Failure	2	2
Ischemic Heart Disease	1	1
Mitral Valve Prosthesis	1	1
Post Cardiac Catheter	1	1
Post Valve Repair	2	2
Valvular Heart Disease	1	1
Central nervous system disease		
Alzheimer	1	1
Dementia	2	2
Meningeomyelocele	1	1
Mental Retardation	1	1
Pulmonary Disease		
Bronchial Asthma	5	5.1
Pleural Effusion	1	1
Oncology disease		
Adenocarcinoma	1	1
Meningeomyelocele	1	1
Acute myeloid leukemia	1	1
Cancer colon	2	2
Liver cancer	1	1
Prostate cancer	2	2
Sarcoma	1	1
Hematological disease		
Pancytopenia	1	1
Sickle cell anemia	3	3.1
Liver disease		
Hepatitis C	3	3.1
Renal disease		
Hypertension	14	14.3
Acute kidney injury	1	1
End stage renal faliure	2	2
Hydronephrosis	1	1
Lupus nephritis	1	1
Renal stone	1	1
Endocrine disease		
Diabetes Mellitus	16	16.3
Hypothyrodism	7	7.1
Thyroidectomy	1	1
Immunodeficiency disease		
Primary immune defeciency	1	1
Sever combined immune defeciency	1	1
DEATH	1	1

DISCUSSION

In this study we noticed an increase in the number of streptococcal isolates that cause bacteremia, meningitis in addition to soft tissue infection over the last few years in our hospital. Viridians Group Streptococci (VGS) is part of the normal flora of the human gastrointestinal and genital tracts, are increasingly recognized as both a frequent and life-threatening cause of infection in children and adults especially in immunocompromised as a result of chemotherapy or hemopoietic stem cell transplant (HSCT). VGS are now the third most common cause of bacteremia in pediatric hematology and cancer patients globally. VGS bacteremia (VGS) may result in the development of Viridans Group Streptococcal Shock Syndrome (VSSS), a toxic shock-like syndrome, characterized by hypotension and/or acute respiratory distress syndrome (ARDS)[13]. Among the VGS group, Streptococcus mitis was the most common isolate in our study ,and the most common isolate(*Streptococcus mitis*) that caused bacteremia ,but cancer was not identify as relevant risk factor. In a study that involved

patients with cancer diagnosed with bacteremia of 118 unique VGS strains causing bacteremia in; Streptococcus mitis (68 patients) and S. oralis (22 patients) were the most frequently identified strains. Compared with patients infected with non–S. mitis strains, patients infected with S. mitis strains were more likely to have moderate or severe clinical disease (e.g., VGS shock syndrome) [14].

Invasive GAS infections have increased worldwide in the recent past, despite the organism remaining sensitive to penicillin and other commonly used beta-lactam antibiotics. In this study Group A streptococci (GAS) was identified to cause a number of bacteremia and soft tissue infection (cellulitis) . The findings from Strep-EURO study confirmed a high incidence of Group A streptococci severe disease in Europe. In that study a total of 5,522 cases with *Streptococcus pyogenes* were identified across the 11 European countries, Skin and soft tissue were the most common foci of infection, with 32% of patients having cellulitis and 8% necrotizing fasciitis. The overall 7-day case fatality rate was 19% [15].

In another study a survey conducted in 194 hospitals, accounting for 51% of acute care hospital admissions in France, a 664 cases of invasive GAS infections was identified with an annual incidence of 3.1 per 100,000 population, the case-fatality ratio was 14% and rose to 43% in the case of streptococcal toxic shock syndrome. Bacteremia without identified focus (22%) and skin/soft tissue infections (30%) were the most frequent clinical presentations [16]. Group B streptococci (GBS) are a leading cause of infections in neonates and pregnant women and also cause invasive diseases in children and non-pregnant adults. Group B Streptococcus (GBS) was the main causative organism of invasive infections in newborns due to vertical transmission from the colonized mothers. A study was undertaken to determine colonization rate, serotype distribution, genotypic characterization, antibiotic susceptibility profiles and molecular characteristics of erythromycin-resistant strains of GBS in pregnant women in Beijing, China. Vaginal–rectal swabs were collected from a total of 2850 pregnant women at 35–37 weeks of gestation, in which 7.1% were GBS positive. Serotypes III, Ia and V predominated. All isolates were penicillin susceptible, whereas the resistance rates for erythromycin and clindamycin were strikingly high [17]. In our study (GBS) was isolated in significant episode of bacteremia. *Streptococcus pneumoniae* is the major cause of serious invasive diseases such as bacterial pneumonia, septicaemia, and meningitis in young children worldwide. An estimated 14.5 million cases of invasive pneumococcal disease (IPD) occurred globally in 2000 before introduction of pneumococcal conjugate vaccines (PCVs). Widespread availability of PCVs has reduced the burden of IPD substantially, from over 800,000 annual deaths before PCV introduction to 541,000 deaths in 2008. Although two formulations of PCVs are available to protect against disease, *S. pneumoniae* still poses a significant burden on individuals and healthcare systems [18].

Similarly in this study majority of the meningitis cases and significant number of bacteremia episode were caused by *S. pneumoniae*.

The susceptibility test for the streptococcal isolates where extremely difficult to be compared to other research since we did not find exactly similar study like this study that studied all different types of the streptococcal isolate , however taking each streptococcal isolate separately and comparing it with other studies , there was no difference in the susceptibility patterns[19].

In conclusion, Taking into account that streptococcal infection represents a significant problem on a global scale, epidemiological surveillance, antimicrobial resistance at the local level could provide important knowledge and help to improve treatment and prevent invasive infection caused by this microorganism. To our knowledge, this is the first study discerning the clinical- epidemiology of invasive alpha and βHS invasive βHS from Riyadh region of Saudi Arabia.

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