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Research Article

Isolation and Molecular characterization of Bacterial Sinusitis from Sudanese Patients

Zolfgar R. Hassan[1], Hanan Moawia Ibrahim[1], Fadwa M.Elsheikh[1] and Nagat Abdalla Elawad[2]

[1]Central Lab. Ministry of Higher Education and Scientific Research, Sudan.

[2]Department of E.N.T. Soba Hospital, Sudan.

ABSTRACT

The study aimed to isolate, identify and characterize the common causative organisms of bacterial sinusitis in Sudanese patients. Throat swabs were taken from postnasal discharge of 100 adult Sudanese patients diagnosed as chronic bacterial sinusitis, males to female's ratio about 1: 2. The organisms were isolated and identified using conventional methods, rabid and standardized API systems and molecular techniques. Pathogenic bacteria were found in 92 samples (92%). Ninety-seven isolates from the samples were obtained, 83 isolates (85%) were Gram-positive bacteria, while 14 isolates (14%) were Gram-negative bacteria. In the isolated Gram-positive bacteria (42%) were *Staphylococcus aureus,* (8%) were *Staphylococcus intermedius,* (10%) were *Streptococcus pyogenes,* (11%) were *Streptococcus equismilis,* (10%) were *Enterococcus faecalis,* (18%) were *Micrococcus varians* and (1%) were *Corynebacterium pseudotuberculosis,* While the isolated Gram-negative bacteria (86%) were *Klebsiella pneumonia sub spp pneumoniae* and (14%) were *Escherichia coli.* Lancefield *Streptococcus* species grouping showed that 8 of the 25 (32%) of *Streptococcus*-suspect strains obtained from this study, were group A (*S. pyogenes*), 9 were group C (36%) (*S. equisimilis*) and 8 group D (32%) (*Enterococcus* spp). The conventional methods for identification were similar to those obtained by the rapid tests kits and API 20 E for *Staphylococci* species*, Streptococci* species and *Enterobacteriaceae* respectively. While molecular techniques gave better results in chronic sinusitis compared with standard culture technique

Key words: Sinusitis, API systems, PCR, *Staphylococcus aureus* and *Klebsiella pneumonia* sub spp *pneumonia.*

INTRODUCTION

Sinusitis is an acute or chronic inflammation of one or more of the paranasal sinuses, the cause of which may be allergic, viral, bacterial or fungal. It can occur at any age and distributed all over the world. Bacterial sinusitis is a secondary infection caused by trapping of bacteria in the sinuses during the course of cold or allergy.

Sinusitis is an extremely common disease process, approximately 0.5-5% of upper respiratory infections are complicated by acute sinusitis[1]. Acute bacterial sinusitis is a common disorder that affects children and adults and can produce potential life-threatening complications[2]. The inflammation can have more than one cause such as nasal polyps, allergic reactions, deviated nasal septum, trauma to the face, respiratory tract infections, immune system cells, allergies such as hay fever and other medical conditions such as the complications of cystic fibrosis, gastroesophageal reflux and, HIV and other immune system-related diseases may also result in nasal blockage[3,4]. Some organisms are present more frequently than others; it is important to consider the patient's age, clinical presentation, and immunocompetence status. In acute bacterial ethmoidal and sphenoidal sinus disease, the most common isolated organisms are *Streptococcus*

pneumoniae, *Haemophilus influenzae*, and *Moraxella catarrhalis*. Most of these infections, however, are preceded by upper respiratory infections caused by viruses. These organisms are also present in other forms of acute sinusitis such as frontal[5]. On the other hand, Gram-negative *Bacilli*, *Staphylococci* sp. and respiratory anaerobes are seen more commonly in chronic sinus infection, which may also be caused by exacerbations of infection with the bacterial species that cause acute disease[6]. Studies have shown that the most common organisms responsible for the development of sinusitis are *Staphylococcus aureus*, *Staphylococcus intermedius*, *Streptococcus pyogenes*, *Streptococcus equismilis*, *Enterococcus faecalis*, *Micrococcus varians*, *Corynebacterium pseudotuberculosis*, *Klebsiella pneumonia* sub spp *pneumoniae* and *Escherichia coli*, however, broader spectrum of possible pathogens, which may be resistant to first-line antibiotic therapy, makes identification of the causative organisms crucial for successful treatment. A large number of studies over several decades have sought to determine the bacterial organisms present in sinusitis. Studies dealing with bacterial findings of sinusitis, which have attempted to define the correct pathogens, have been frequently published, but have reported a diversity of results. Although Sudan is a tropical country and has different climates, sinusitis has not taken much concern in the Sudanese literature, but during clinical practice we observed an increase in the number of patients presented with sinusitis in Ear, Nose, and Throat clinics. Patients with sinusitis were 32% of all patients with nasal diseases and affect females more than males with the highest incidence at the ages 21-30, the majority of patient were curried with medical treatment by the appropriate antibiotics/antihistamines and local decongestant[7]. Affection of males in the middle age groups has a negative impact in the national economy especially when they use an antihistamine which causes fatigue and dizziness. Diagnosis of sinusitis is mainly based on clinical detection[8]. Due to the high prevalence of sinusitis in Sudan, especially in Khartoum province, in this study, bacteriological survey of patients with chronic sinusitis were done in order to help physicians in choosing better antibiotics for the empiric therapy of sinusitis

MATERIAL AND METHODS

This prospective study was performed at the Bacteriology Lab. of Central Laboratory. The study conducted in Khartoum E.N.T. Hospital and E.N.T. Department, Soba University Hospital. Patients of 15-60 years old presenting with recurrent or persistent sinus complaints, including nasal discharge, nasal obstruction, cough, headache, facial fullness, change in taste or smell, and frequent throat clearing and/or wheezing were evaluated for chronic sinusitis. Only patients who had not received any antibiotic treatment at least two weeks before the collection of specimens were included in the study.

Sample collection

Throat swabs were taken from postnasal discharge of 100 adult Sudanese patients diagnosed as chronic bacterial sinusitis using sterile cotton tipped swabs. The samples were taken from postnasal discharge after depressing the tongue under complete aseptic condition and transported immediately to the microbiological laboratory using Stuart Transported Media in an ice container. The study improved by ethical committee and the consent was taken by patient using questionnaire.

Microbiological analysis

The swabs were cultured on Blood Agar, Chocolate Agar, and Mac Conkey Agar media and incubated aerobically, an aerobically with Co_2 at 37°C. The growth characteristic of bacteria in artificial media is often essential for identification. It includes colonial shape, size, colour and growth pattern. Consistency and haemolysis are useful criteria. The organisms were isolated and identified using conventional methods[9]. Gram stains reaction, morphological and biochemical tests. Rabid and standardized API systems, API 20E (bioMerieux, inc.France), HiStaph and HiStrep kits (himedia , India) were used as confirmatory method for the selected isolates .

Molecular assay:

DNA was extracted from all pure cultures of *Staphylococcus aureus* isolates, a loop-ful of the culture was suspended in 0.5 ml of distilled water. DNA extraction was performed using a commercial kit (Qiagen, Germany)[10]. The extraction was performed as per the manufacturer's instruction. The PCR reaction was performed in a total volume of 25µl. containing 4 µl of DNA, 0.25U Taq DNA polymerase (Vivantis, Malaysia). , 5 µl of 10 x PCR amplification buffer, 2µM primer (Gene bank database accession No AJ133520). A26-nucleotide forward primer, GF-1(5-ATGGTTTTGGTAGAATTGGTCGTTTA-3), corresponding to positions 22 to 47 of the gap gene, and a 25- nucleotide reveres primer,GR-2(5-GACATTTCGTTATCATACCAAGCTG-3) (Prizma, Istanbul). 0.5 mM deoxynucleoside triphosphate and double distilled water to a final volume of 25µl. The cycle conditions were followed by 40 cycles denaturation at 94°C for 1 min, primer

annealing at 58°C for 1 min and DNA extension at 72 °C for 1.5 min. After the final cycle, the reactions were terminated by an extra run at 72 °C for 10 minutes. The PCR product was visualized by electrophoresis at 120 v for 30 min on 1.5 % agarose with 0.5 mg / ml ethidium bromide and photographed with the aid of gel documentation system (Bio Rad, USA).

RESULTS

In this study throat swabs were taken from postnasal discharge of 100 adult Sudanese patients diagnosed as recurrent bacterial sinusitis, their ages range between 15-60 years old with mean age 36.3 ± 15.7 SD for males and 28.7± 10.4 for females. 32 patients were males and 68 patients were females, males to female's ratio about 1: 2. Pathogenic bacteria were found in 92 samples (92%), while no growth was obtained from 8 samples (8%). Ninety-seven isolates from the sample were obtained, 83 isolates of them were Gram-positive bacteria, while 14 isolates were Gram-negative bacteria.

In the isolated Gram-positive bacteria (42%) were *Staphylococcus aureus*, (8%) were *Staphylococcus intermedius*, (10%) were *Streptococcus pyogenes*, (11%) were *Streptococcus equismilis*, (10%) were *Enterococcus faecalis*, (18%) were *Micrococcus varians* and (1%) were *Corynebacterium pseudotuberculosis*, (Figure 1). While the isolated Gram-negative bacteria (86%) were *Klebsiella pneumonia* sub spp *pneumoniae* and (14%) were *Escherichia coli*, (Figure 2).For further confirmation, HiStaph and HiStrep kits were used for identification of *Staphylococcus* species and *Streptococcus* species, while API 20E was used for *Enterobacteriaceae* identification (Figure 3) respectively. In Polymerase Chain Reaction technique (PCR) 933pb fragment was obtained from DNA of all the isolated *Staphylococcus aureus* (Figure 4). Lancefield *Streptococcus* species grouping showed that 8 of the 25 of Streptococcus-suspect strains obtained from this study, were group A (*S. pyogenes*), 9 were group C (*S. equisimilis*) and 8 group D (*Enterococcus spp.*) (Figure 4).

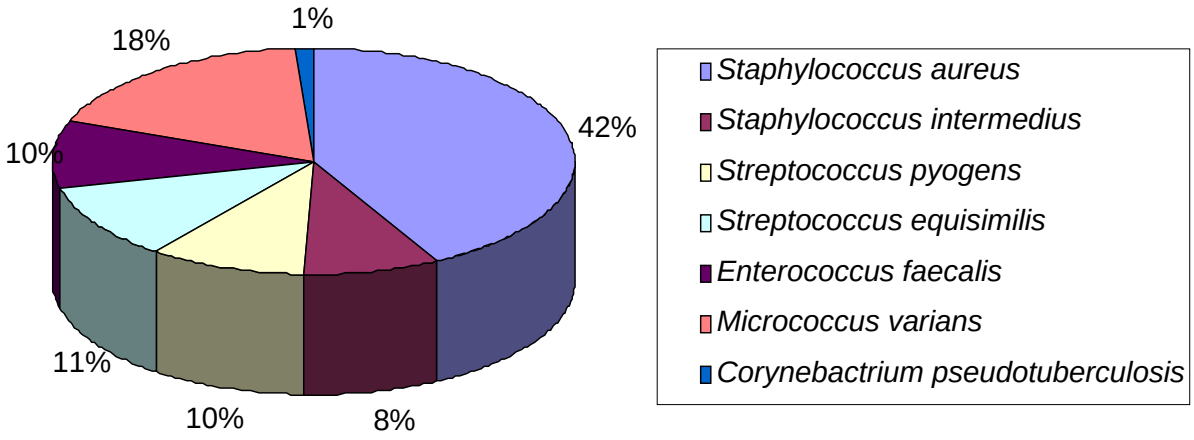


Figure 1
Gram-positive bacteria species isolated.

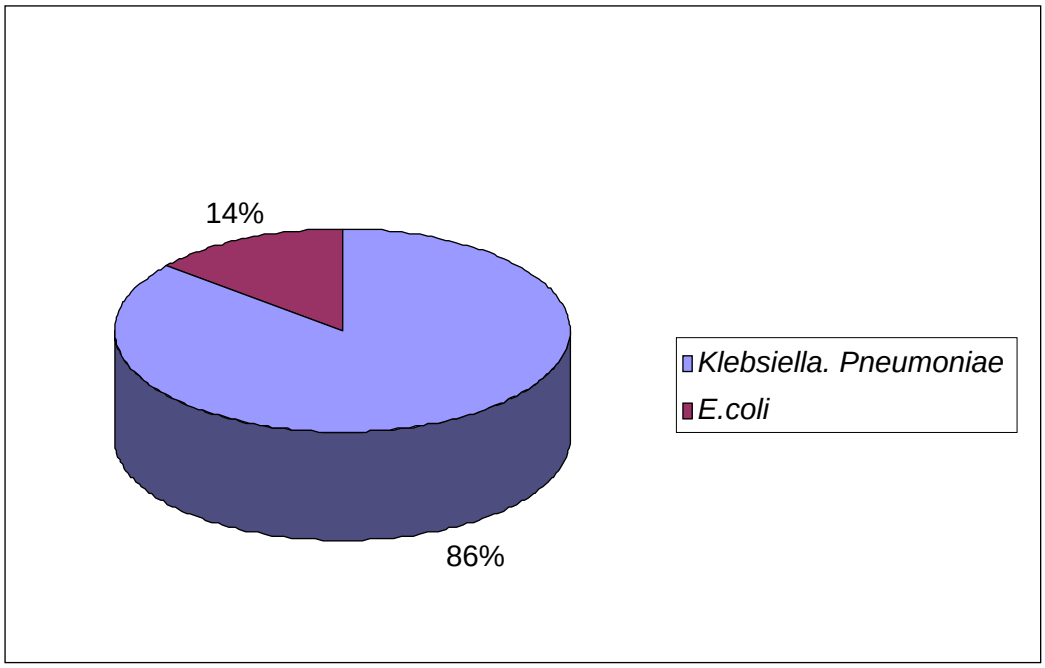
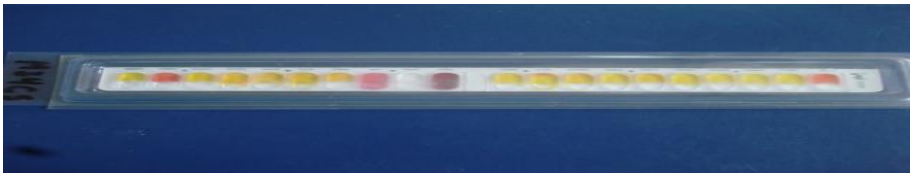


Figure 2
Gram-negative bacteria species isolated.

(A)

(B)



(C)

Figure 3
HIStaph *Staphylococcus aureus* (A)
HIStrep *Streptococcus pyogenes*(B)
Analytical profile index API 20*Enterobacteriaceae*(C).

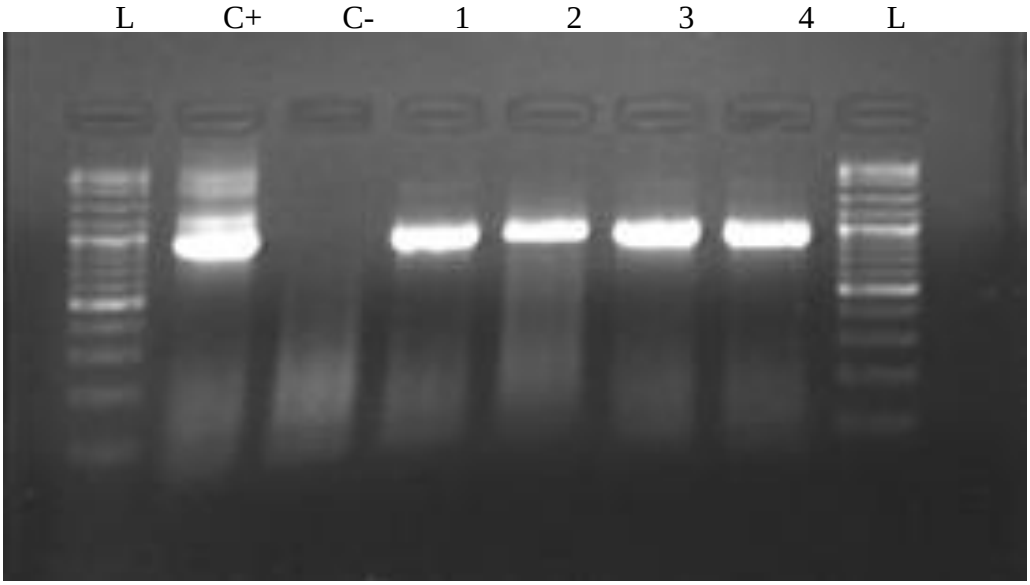


Figure 4
Agarose gel electrophoresis of 933bp PCR amplification product using primers GF 1 and GR2 L: Ladder. C+: positive control. C-: negative control, 1-4. *Staphylococcus aureus.*

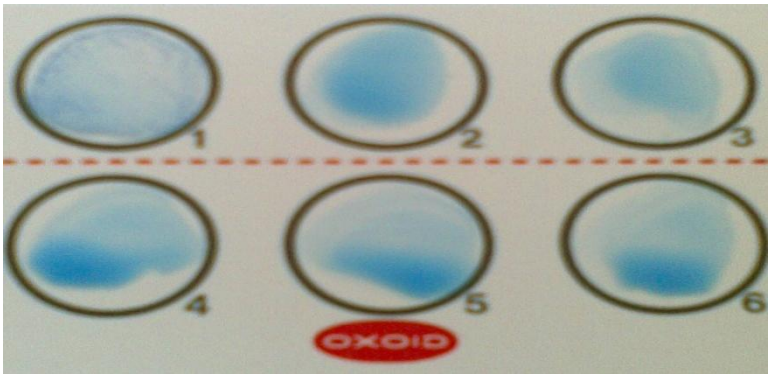


Figure 5
Lancefield Streptococcus species group (A, B, D).

DISCUSSION

Sinusitis is one of the most frequent medical complaints, and is estimated to affect about one in eight persons throughout their life time[11]. Treatment of chronic sinusitis is complicated due to increase of antibiotic-resistant bacteria. 60% of sinusitis in cases of community acquired patients yield bacteria, viral illness may be the most common triggering mechanism for sinus disease. Sinusitis is the most prevalent disease for adults diagnosed in ambulatory medical care and is treated with antibiotics[12]. In Sudan, one clinical review reported sinusitis in 32% of all patients with nasal diseases[7]. While our study reported (90%) of patient with bacterial sinusitis this may be due to environmental factor like hot dusty weather. In this study we noticed that the ratio of female infection is higher than male about 2:1 this finding in accord with the result of Chen et al[13,14,15]). Similar ratio was reported by Stankiewiics[16] but different result was obtained by other studies[17],

whom they found that males were affected more than females in a ratio of 7:1 and also disagree with the result of Yagi[7], in his retrospective study in the patterns of sinusitis in Sudanese patients. This difference in the pattern of sinusitis in Sudanese ten years ago can be attributed to that patients were housewife with minimal exposure to outdoor environmental contamination while in this study most of our patients were worker with daily exposure to hot dusty weather. There is variability in the isolated bacteria in our study, *Staphylococci* were isolated from about 50% of studied was finding in according to the result of Gundes *et al.,*[18] who found *Staphylococcus spp.* accounted for more than 50% of the cases studied, also similar result was obtained by Yagi[7] who found that *Staphylococci*, *Streptococci* and *E. coli* were the commonest causative organisms isolated in Sudanese patients with sinusitis. *S. aureus* was a common pathogen isolated from about 42% of all cases in this study, this result is similar to the result obtained by[15,19], Other study[20] reported that *S. aureus* and coagulase negative *Staphylococci* as a common pathogen and similar to the result of Brook[21] who found *S. aureus* as a common pathogen in sphenoid sinusitis. In the present study *S. aureus* play the main role in bacterial sinusitis infection in Khartoum state. These results disagree with the result of Tellez. *et al*,[22] who found *H.influenzae* as the common pathogen.The predominance of co-agulase-negative *Staphylococci. S. aureus* and other Staphyloccoi species may occasionally cause sinusitis in adults and children[23], while study in Iran found that the most common bacteria found in the nasopharynx were Gram-positive *Bacillus, coagulase* negative *Staphylococcus* and *Staphylococcus aureus* with rates of 20%, 16% and 15% respectively and bacteria isolated from opening sinus were Gram-positive *Bacillus* 24%, *Enterobacter aerogenes* 10%, coagulase negative *Staphylococcus* 18% and *Staphylococcus aureus* 19%[24]. The most detectable rate of Methicillin-resistant Staphylococcus (MRS) bacteria isolated were Gram negative bacteria 39(76.47%) and *Enterobacter* spp[25]. These differences in the rates of the recovered pathogens might be due to geographical differences and or differences in the sanitary and hygiene measures adopted in each country. The high dominance of *Staphylococci* due to their presence as a part of the normal flora of the upper respiratory tract and also they are considered as skin commensally that can cause opportunistic infection at site, if the host resistance was lowered such as in cases of skin damage or mucous membranes abrasions[26]. Most of the publications reviewed showed that *Streptococcus* species were most frequently isolated but with variable species and percentage. In this study the total percentage of *Streptococcus* species was (21%). This percentage is less than that of Gundes *et al.,*[18] who found *Streptococcus* spp represent (24 %) of the total isolates from the middle meatus. The results disagree in the type of the isolate predominated, *S. pneumoniae* was (8%) and *Streptococcus viridians* (10%) while in this study *S. pyogenes* represented (11%) and *S. equisimlis* (10%) of the isolated. The small parentages of *S. pyogenes* in this study agree with Poole[27,22]. While one study evaluated the human nose habitats bacterial and they found out that S.aureus was the one found most frequently in association with Staphylococcus epidermidis[28]. Gram-negative bacteria were less common pathogens in sinus infection in this study, *K. pneumoniae* and *E. coli* were the only two species isolated. *K. pneumoniae* was predominating over *E. coli*, it showed (86%) and (14%) of the total Gram-negative bacteria, respectively. These results agree with Busaba *et al.*[14] who obtained these isolates from chronic ethnocide sinusitis but in smaller percentage and disagree with other report who reported the usual pathogens are the enteric organisms such as *K. pneumoniae*, *Pseud. aeruginosa, Enterobacter* spp and other Gram-negative bacteria[29]. Compared with other study carried out in Omdurman isolated *Moraxella catarrhalis, Staphylococcus epidermidis, Stenotrophomonas maltophilia, Pseuedumonas* spp. and *Bacillus polymyxa.* Which were not found in this study[15]. Classical physiological and biochemical tests are not adequate to analyses and rapidly identify bacteria from microbial communities. Because the bacterial populations involved often have similar nutritional requirements and grow under similar environmental conditions[30]. The results obtained in the present study using the conventional methods for identification were similar to those obtained by the rapid tests such as Histaph, Histrept kits and API20E for *Staphylococci* species, *Streptococci* species and *Enterobacteriaceae* respectively. The analysis of 16S rRNA gene sequences has been the technique generally used to study the evolution and taxonomy of *Staphylococci.* However, the results of this method did not correspond to the results of polyphasic taxonomy, and the related species cannot always be distinguished from each other. Thus, new phylogenetic markers for *Staphylococci* species were used[31,32]. There is a wide variety of molecular techniques used for microbial identification such as polymerase chain reaction (PCR) with specific primers[33]. PCR method gave better results in chronic sinusitis compared with standard culture technique[34]. The *gap* gene of *S. aureus*, encoding glyceraldehyde-3-phosphate dehydrogenase, was used in the present

study as a target gene for *Staphylococcal* isolates from sinusitis. The result of API obtained was similar to the result of Yuguerose *et al.,*[35]. The presence of gap gene indicated that *Staphylococci* are pathogenic. However, this gene was not detected in some pathogenic species of *Staphylococci*.

CONCLUSION

Throat swabs were taken from postnasal discharge of 100 adult Sudanese patients diagnosed as chronic bacterial sinusitis. The result revealed that (42%) were *Staphylococcus aureus*, (8%) were *Staphylococcus intermedius*, (10%) were *Streptococcus pyogenes*, (11%) were *Streptococcus equismilis*, (10%) were *Enterococcus faecalis*, (18%) were *Micrococcus varians* and (1%) were *Corynebacterium pseudotuberculosis*, (86%) were *Klebsiella pneumonia sub spp pneumoniae* and (14%) were *Escherichia coli*.

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