Sud Med Lab J, issue1, vol 3: 30-37

OriginalArticle

Sudan Medical Laboratory Journal

Isolation and Identification of Streptococci from sore throat in Khartoum E.N.T. Teaching Hospital

Ibrahim, A.E[1]. Elsanousi, S.M[2].

[1]*Pathology Department,Khartoum University, Faculty of Veterinary Medicine*
[2] *Microbiology Department, Khartoum University, Faculty ofVeterinary Medicine*

Abstract

Objectives:This study aimed to isolate and identify *Streptococcus pyogenes*and to determineits prevalence in patients whoattended Khartoum Ear Nose Throat TeachingHospital suffering from sore throat, (Tonsillitis – Pharyngitis- Laryngitis).

Methods:One hundred and fifty three throat swabs were collected from the patients regardless of their age. All swabs were cultured on 5% blood agar plates for overnight. Colonial morphology and type of haemolysis were observed upon diagnosis but they were not used as stringent criteria for diagnosis. All isolates were subjected to further examination includingGram's stain and biochemical reactions (Catalase, bacitracin sensitivity, CAMP reaction, aesculin hydrolysis, arginine hydrolysis, and hippurate hydrolysis Optochin sensitivity, growth on 10% and 40% bile and Bile solubility). These tests were done for (Gram positive catalase negative organisms),in addition to Lancefield grouping andsensitivity to antibiotics.

Results:The results showed that not only *Streptococcus pyogenes* was incriminated in this type of infection, as group F was also isolated.

ConclusionsWe concluded that,in addition to *S. pyogenes*other groups can also cause sore throat and further examination should be made when Group A Streptococci(GAS) is not detected in patients suffering from sore throatso as to reduce the use of incorrect antibiotics and recurrent infections.

Correspondence address: Abbas Elsafi Ibrahim, mail: *Abbasesafi@gmail.Com*

Pathology Department, College of Veterinary Medicine, Khartoum University, P.O. Box 32 Khartoum, Sudan

Introduction

Bacterial infections are significant threat to the health of neonates and adults. The throat and skin of the human host are the principal reservoirs for the bacterial pathogen *Streptococcus pyogenes* strains (commonly referred to as group A streptococci (GAS) which are among the most prevalent of pathogenic bacteria which affect humans. They have a worldwide distribution, and humans are the only known biological host. *S. pyogenes* can cause a life-threatening illness, such as toxic shock syndrome, or trigger autoimmune disorders, such as rheumatic fever. However, most often *S. pyogenes* causes only mild infections at either the throat or skin, leading to pharyngitis or impetigo, respectively. These two tissue sites serve as the principal reservoir for organisms of this species (1).The sore throat decision rule can identify both patients who are so likely to have GroupA Beta Haemolytic Streptococci (GABHS) that a confirmatory test is not needed and patients who are so unlikely to have GABHS that further testing is unrewarding. Using the rule will successfully identify most patients who need treatment for GABHS infection, while decreasing antibiotic use for sore throat by about 80%. (2). The 4 most useful features to look for in diagnosing GABHS are enlarged sub-mandibular glands, throat exudates, fever, and absence of cough and runny nose (2).Bacterial sore throats respond well to antibiotics, whereas viral ones do not. However, sore throat remains a leading cause for physician visits, and researchers have long struggled to determine how best to treat it. The best clinical management of patients with sore throat depends on both the clinical probability of group A streptococcal infection and clinical judgments that incorporate the importance ratings of the individual patients as well as practice circumstances(3).Management of pharyngitis is commonly based on features which are thought to be associated with Lancefield group A beta-haemolyticStreptococci (GABHS) but it is debatable which features best predict GABHS. Non-group A strains share major virulence factors with group A, but it is unclear how commonly they present and whether their presentation differs(4). A study concluded that groups C and F streptococci were implicated as a cause of acute pharyngitis in 6.2% of the specimens among other groups of Streptococci. Most of these isolates have the ability to produce more than one virulence factor. There was a

high rate of resistance among isolates for β-lactam antibiotics; however, they were highly susceptible to vancomycin, ofloxacin, and clindamycin (5). Adult group C beta-hemolytic streptococcal pharyngitis has a prevalence of approximately 5% and can present with a broad spectrum of severity. Negative rapid test was observed in a woman with severe pharyngitis. (6). This presentation illustrates the importance of a systematic approach to evaluating patients with negative rapid strep tests and worsening pharyngitis. Current guidelines assume that group A beta-hemolytic streptococcus is the only important treatable cause of episodic pharyngitis. These guidelines dissuade physicians from using antibiotics, unless a firm diagnosis of group A beta-hemolytic Streptococcus can be made. The study presents a woman who presented with worsening pharyngitis and negative rapid antigen tests for group A pharyngitis. This presentation raises important diagnostic considerations for the General Internist (6).The role of large colony Streptococci groups C or G as pathogen agentsin sore throat has been questioned;a study aimed to analyze clinical features of patients with large colony Streptococci groups C or G compared with patients with group A Streptococci (GAS) and with negative cultures. The result of the study showed that out of 306 patients with a sore throat, 244 were adults and 62 were children under 10 years old; 40% were men. One hundred and twenty-seven had GAS, 33 had Streptococci groups C or G, and 146 had negative throat cultures. It was concluded that patients with tonsillitis caused by *Streptococcus* groups C or G have, to a large extent, the same clinical picture as patients with GAS. Large colony streptococci groups C and G should be considered as throat pathogens in line with GAS (7).The objectives of this study are to isolate and identify Streptococci in patients suffering from sore throat, to identify the groups of Streptococci by biochemical and serological methods and to study their susceptibility to antibiotics.

Materials and methods

Throat swabs were taken from 153 un-treated adults and children a tKhartoum Ear Nose Throat Hospital irrespective of their age. The case definition of sore throat is diagnosed by looking for The 4 most useful features: enlarged sub-mandibular glands, throat exudates, fever, and absence of cough and runny nose(2),Patients who were under treatment with antibiotic or treated seven days before

collection were excluded. A pre-coded questionnaire on medical, social and family history and antibiotic consumption was completed after obtaining informed verbal consent.

Procedure

Swabs were then streaked on the plates of the blood agar media and the plates were incubated under 10% Co2 over night at 37ºC. Defibrinated sheep blood was used in preparing blood agar medium in a concentration of 5% assuming that it was free from anti microbial agents and fresh. It was used for the primary identification, type of haemolysis, bacitracin and optochin sensitivity and the CAMP reaction. Bile salts solution was prepared in a concentration of 10% solution and used for the bile solubility test. Nutrient broth medium was used for the catalase test after adding 5% serum. Aesculin broth medium was used for Aesculin test, Arginine broth and **Nessler's Reagent** were used for Arginine test and Hippurate broth medium was used for detection of Hippurate hydrolysis. Throat swabs were collected from the specific patients who attended Khartoum ENT Teaching Hospital. The type of haemolytic reaction displayed on blood agar was used to classify the streptococci to α, β or non-haemolytic streptococci. The gram stain was done from the overnight growth and gram-positive reacted organisms were subjected to further examinations including different biochemical tests which were done as described by Barrow and Fellthan, (2003) (8) including: Catalase test (streptococci are considered as catalase-negative. Catalase-positive samples were excluded), CAMP reaction, Aesculin test, Arginine test, Growth on bile, Bile solubility, Hippurate hydrolysis, Bacitracin sensitivity (used to differentiate group A streptococci from other groups) and Optochin sensitivity. Lancefield grouping for serologic identification of the organism groups was made by using the commercial grouping antisera and was done as described by the manufacturers. Sensitivity to antibiotics was done by the disc diffusion method.

Results

The number of Streptococci isolated from tonsillitis and pharyngitis was 115 samples out of 153 samples. Thirty eight samples showed catalase-positive reaction and were excluded. Nine samples were isolated from the pharynx (table, 1). The grouping of the organism included in the study according to biochemical

reaction and type of haemolysis (catalase negative organisms) resulted in recognition of two groups A &F. The most prevalent Streptococci isolated were *S. pyogenes* which was isolated from 94 samples out of 115 samples (81.74%). Fifteen samples (13.04%) shared characters of group A & C, 4 samples (3.48%) were group F, while 2 samples (1.74%) could not be clearly grouped according to biochemical reaction as they showed bacitracin sensitivity but the type of haemolysis(α) and the bile solubility is not a criteria of group A. They differed from *S. pneumoniae*as they showed bile solubility but they were Optochin-negative as shown in (table, 2).Grouping by Lancefield kit results in recognition of two groups (A & F only), and the two mentioned organisms did not react as shown in(table, 3).The sensitivity to antibiotics was done to all sample included and their results were shown in form of crosses. Group AStreptococci was found highly sensitive to penicillin, moderate sensitivity to fusidic acid, augamentin and vancomycin. Other antibiotic showed mild sensitivity or even resistant, while group F were found highly sensitive to vancomycin, with moderate sensitivity to penicillin, fusidic acid and augamentin with mild sensitivity to gentamycin and complete resistant to the other antibiotics. GroupA or Cand not clearly grouped organisms were found almost similar to group A except resistant to ciprofloxacin and gentamycin as shown in table 4.

Table (1): Infection rate with Streptococci in examined patients

No examined	Gram + ve	Gram – ve	Catal. – ve	Catal. + ve	Tonsillitis	Pharyngitis
153	153	zero	115	38	106	9

Table (2): grouping according to biochemical reactions

Biochemical reaction	Group A	Group A or C	Group F	non typable
Typeof haemolysis	β94 (81.74%)	α 15 (13.04%)	β4 (3.48%)	α 2 (1.74%)

Camp reaction	-	-	-	-
growth on 40% bile	-	-	-	-
Bile solubility	-	-	-	2
Aesculin	67	11	4	-
Arginine	94	15	4	-
Bacitracin	94	15	-	2
Optochin	-	-	-	-
Hippurate	-	-	-	-

Table (3): Grouping according to Lancefield grouping kit

Group	A	B	C	D	F	G	Non
No	109	-	-	-	4	-	2

Table (4): Antibiotics sensitivity of bacteria isolated from sore throat

Antibiotic / unit	Group A	Group F	Group A or C	non typable
Penicillin- G10	xxxx	xxx	xxxx	xxx
Fusidic acid 10	xxx	xxx	xxx	xx
Augamentin30	xxx	xxx	xx	xxx
Vancomycin 30	xxx	xxxx	xx	xxx
Gentamycin 10	x	x	x	-
Ciprofloxacin 5	x	-	-	-
Chloramphenicol 30	x	-	-	x
Erythromycin 10	x	-	x	x

Discussion

S. pyogenes is a major pathogen capable of causing a wide range of diseases in different age group of people (10). The prevalence of the carriage of GAS was more common than expected group G and group C were also isolated from throat *S. pyogenes* is a major pathogen capable of causing a wide range of diseases in different age group of people (10). The prevalence of the carriage of GAS was more common than ext cultures (11). In the present study there were 15 organisms which could not be clearly grouped by biochemical reactions into group A or Group C because they showed bacitracin sensitivity, which is related to group A, and type of haemolysis similar to group C which is bacitracin negative.Our results are similar to those of Alaa*et al.*(5)in isolating group F Streptococci as a cause of sore throat together with group C, but we could not identify group C. The results also agree with Graham (2)) and Dalalah*et al*. (3) in proving that the proper identification of the groups is necessary for the correct use of antibiotics. The present findings seem to differ from the results of Mobin*et al.* (6) who isolated only group C streptococci as a cause of pharyngitis in a recurrent case and Morten *et al.*(7) who isolated group C and group G as a cause of sore throat. Recent studies in America (5), and as shown also in the present study, proved that there are many groups of streptococci that can cause sore throat including group F, C, and G β-haemolytic streptococci. Ignoring these groups in the diagnosis will lead to misuse of antibiotics and recurrence of infections after a short period of antibiotic treatment resulting in resistant bacteria. In our study, we found that most of the isolated respond well to antibiotics as Dalalah (3) found that sore throat bacteria respond to antibiotics. Sore throat infection was mostly incriminated to *S. pyogenes* (GAS)and the diagnosis depended only on symptoms and physical examination of the tonsils and pharynx. Laboratory investigations were rarely requested and include only ASO titer, ESR and CBC. Bacteriological investigations if requested, includes bacitracin sensitivity only which is a stringent criteria for GAS.

Conclusion

Most of the bacteria isolated in this study were sensitive to the commonly used antibiotics but, we strongly recommend that a full bacteriological examination must be done as a routine practice for diagnosis of sore throat infections because non-group A strains commonly cause streptococcal sore throats, and

present with similar symptomatic clinical features to group A Streptococci in order to reduce the misuse of antibiotics and repeated infection in our hospitals.

References

1. Awdhesh, K. Brain, G. Spratt, M. C. and Enright, D. E. (2002). BessenSeparation on the Population Genetics Structure of the pathogen *Streptococcus pyogenes.* Infect Immun. 70(4): 1971- 1983.
2. Graham, J. and Worrall.(2007). Acute sore throat Can Fam Physician. 53(11): 1961–1962.
3. *Dalalah, D. and Magableh, S. (2008). A remote fuzzy multicriteria diagnosis of sore throat. Telemed J E Health. 14 (7): 656- 665.*
4. Little, P.Hobbs, FD.Mant, D.McNulty, CA.Mullee, M.PRISM.(2012) Incidence and clinical variables associated with streptococcal throat infections: a prospective diagnostic cohort study. Br J Gen Pract. 62(604):787-794.
5. Alaa, H. Al-Charrakh.Jawad, K. T. Al-Khafaji and Rana, H. S. Al-Rubaye. (2011) Prevalence of β-hemolytic groups C and F streptococci in patients with acute pharyngitis. N. Am J med Sci. ; 3 (3): 129 – 136
6. Mobin, S. Robert, M. Centor, M.J. (2007) Severe Acute Pharyngitis Caused by Group C Streptococcus. J Gen Intern Med. ; 22 (2) 272-274
7. Morten, L. Ernst, A.Høiby, P. H. (2005). Clinical symptoms and signs in sore throat patients with large colony variant β-haemolytic streptococci groups C or G versus group A . Br J Gen Pract. ; 55 (517) : 615 – 619.
8. Barrow, G. I. and Fellthan, R. K.A. (2003) Cowan and Steel's Manual for Identification of medical bacteria, sixth edition.
9. Ray, D. Banerjee, S.Bhattacharya, S. Sinha, S. Bandyopadhyay, D. Ghosal, C. Gupta, S. ;Majumdar, P. K. Saha, S. ;Gupta, S. andBhattacharya, B. (2010). A hospital-based study to evaluate the incidence pattern of group A.streptococcal throat infections from different age group patients.J Indian Med Assoc.108 (2): 81-83.
10. Steer, A. C. Jenny, A. W. Kado, J. Good, M. F.

Batzloff, M. Magor, G. Ritika, R. Mulholland, K. E. and Carapetis, J. R. (2009). Prospective surveillance of streptococcal sorethroat in tropical country.Pediator Infect Dis. 28 (6): 477-482.