Original Research Article

The Prevalence of Group A *Streptococcus* as a Re-Emerging Microorganism in Port Harcourt Metropolis

ABSTRACT

Streptococcus pyogenes is a Group A B-haemolytic streptococcus, responsible for pyogenic infections especially among children. Its diagnostic detection has been a challenge especially in hospitals with limited resources. The aim of this study was to seek the prevalence of *Streptococcus pyogenes* among patients attending University of Port Harcourt Teaching Hospital. This study was hospital based with total of 100 (one hundred) throat swabs examined. The methods and analyses used include crystal violet blood agar culture, bacitracin sensitivity testing, Gram stain, catalase test and microscopic examination. This study found that 5% of throat swab examined detected *Streptococcus pyogenes* from children between the age of <1 – 25 years. The isolates classified as *Streptococcus pyogenes* were only those that grew on crystal violet blood agar, which is gram positive and catalase negative cocci. This present study has shown that with proper diagnostic tools and procedures, *Streptococcus pyogenes* exist in the study area and this should be treated as public health issue of great concern.

Key words: Group A Streptococcus, Pyogenic infection, Prevalence, Re-emerging.

Introduction

Streptococcus pyogenes comprises of Lancefield groupA *Streptococci*. It is a Gram positive bacterium, aerotolerant, extracellular bacterium, consisting of non-motile and non-sporing cocci. It is the main bacterium that causes *Streptococcal* sorethroat. Lancefield group A β-haemolytic *Streptococcal* are associated with the following disease conditions; scarlet fever, pharyngitis, erysipelas [1]. *Streptococcuspyogenes* causes a wide variety of systemic infections including infections of upper respiratory tract and the skin.Its co-infection with *Staphylococcusaureus* is responsible for cellulitis [2]. Its infection is also accounted with acute rheumatic fever (ARF) and acute glumerulonephritis. Infection with *Streptococcuspyogenes* has re-emerged as vital cause of toxic shock syndrome (TSS) and fatal skin and soft tissue infections most especially necrotizing fasciitis [2].

Lancefield method of serologic grouping is the standard critrium for identification of *Streptococcus*species pathogenic human. This technique is based on antigenic difference in group specific polysaccharides present in the bacteria cell wall. There are more than 20 serologic groups so far identified and designated by letters such as A, B, C. Group A strains can be identified via latex agglutination, enzyme immunoassay techniques or coaglutination [2]. Group A strain can be differentiated from the other groups by their sensitivity to the antibiotics known as bacitracin. A 80 – 90% of Non-Group A strains are resistant to bacitracin while the growth of over 95% of the Group A strain the inhibited by 0.411 of bacitracin [3]. On blood agar culture plates, a zone of complete haemolysis (beta haemolysis) is observed. This is typical of *Streptococcus pyogenes* [4].

This bacterium is rarely considered as part of normal human flora and thus handled with caution whenever encountered or isolated [5]. *Streptococcus pyogenes* inhibits skin and upper respiratory tract of humans although it is not considered as part of human biota but may be carried on nasal, pharyageal and sometimes annal mucosa. It is transmitted from person to person through direct contact with the mucosa or secretions or by direct contamination of droplets produced by cough or sneeze. Once exposed, the recipient may become colonized, with subsequent development of the infection [6].

Streptococcus pyogenes releases some virulence factors which made it the most aggressive pathogens isolated from clinical microbiology laboratory. The factors include streptolysin O and S that have dual functions of contributing to virulence and also cause beta-haelolytic pattern on blood agar plates; a guide for its identification [7].

This bacterium causes both localized and systemic infections. Localized infections include acute pharyngitis, skin infections such as erysipelas and impetigo. Other virulence factors include protein F, M protein, streptokinase, DNase, hyaluronidase and streptococcal pyrogenic exotoxins. All these factors have their specific functions in the pathogenesis of *S. pyogenes* [5].

Materials and methods

This study was done in the capital city of Rivers State popularly known as Garden city Port Harcourt. The city covers an area of about 369 kilometer squares. The study was done in the Medical Microbiology Laboratory unit of University of Port Harcourt Teaching Hospital.

Study design: The study was carried out among randomly selected 86 patients between the age of < 1-25years, both sexes attending University of Port Harcourt Teaching Hospital, Rivers State. Work was a hospital based study carried out for period of 3 months. Only patients who presented

themselves to the University of Port Harcourt Teaching hospital, examined and specimens collected by a Doctor were selected. Only freshly collected samples were considered. Both males and females of not more than 25 years of age were included in this study. The specimen used in this study was throat swab without saliva contamination. The materials/ reagents used in this study are sheep blood, throat swab sticks, petri-dishes, clean glass slides, hydrogen peroxide solution, test tubes, glass rod, incubator, weighing balance, microscope, autoclave, water bath, staining rock and immersion oil.

Sample/ Specimen Analysis: The samples collected were analysed by culture method using crystal violet blood agar; sensitivity to bacitracin, catalase test, and gram staining technique.

Inoculation on the Crystal Violet Blood Agar: Nutrient agar was prepared according to manufacturer's instruction **.**Crystal violet (5ml of 0.02%) was added to 500ml nutrient agar previously prepared.The content was autoclaved at 121 degree Celsius for 15 minutes.The solution was transferred to water bath and allowed to cool to 50 degree Celsius. 25ml of sheep blood was aseptically added and mixed gently to avoid air bubbles**.** The crystal violet blood agar solution was poured on petri dishes (15ml each). The plates were allowed to solidify. Throat swab was inoculated on the plates, labeled, placed in a canister and incubated at 37^0C for 24 hours

Inoculation on Blood Agar and Sensitivity to Bacitracin Disc: Adding a bacitracin to a plate of blood agar or any other selective medium is a useful method for detecting *S. pyogenes*. Most strains are sensitive to bacitracin. Other non-group A (group B, C, G) may occasionally show sensitivity to bacitracin. Hence this technique is not confirmatory. Nutrient agar was weighed and prepared according to manufacturer's instruction**.** The agar medium was sterilized by

autoclaving at 121 degree centigrade for 15 minutes. The agar medium was transferred to water bath and allowed to cool to 50 degree centigrade. Sheep blood (25ml) was added and mixed gently well. The blood agar was dispensed on sterile petri dishes and allowed to solidify. Discrete colony from selective medium (crystal violet blood agar) was sub cultured/ inoculated on blood agar. 0.05 units of Bacitracin disc was added on each inoculated plate, placed on the Canister and incubated at 37 degree centigrade for 24 hour. The plates were examined for zone of inhibition of growth caused by Bacitracin

Statistical Analysis: Data obtained was analysed using simple percentage and presented in tables.

RESULTS

There were 100 (one hundred) patients used in this study, comprising of 45 males and 55 females between the ages of < 1 – 25 years who were queried for some throat infections.

Culture of swabs from the throat yielded *Streptococcus pyogenes* in 5 patients within the age range of < 1 – 25 years. The study was covered for period of 3 months and the prevalence rate recorded was 5%, this can be seen in Table 1.

Table 1 Shows the Percentage Occurrence of *S. pyogenes* from Throat Swab Based on Age.

Age Group	M	F	Percentage
< 1 – 5	2	-	2%
6 – 10	1	-	1%
11 – 15	-	1	1%
16 – 20	1	-	1%
21 – 25	-	-	-
Total	**4**	**1**	5%

Table 2 Shows the Distribution of other Organisms Isolated from Throat Swab and their Gram Reactions

Organism	Gram Reaction	No. Isolated	% Occurrence
α-Haemolytic *Streptococci*	Positive cocci	07	8.2
Staphylococcus aureus	Positive cocci	19	22.1
Klebsiella spp	Negative rod	05	5.18
Other B-haemolytic *Streptococci*	Positive cocci	06	6.96
Pseudomonas spp	Negative rod	04	4.65
Candida spp	Not applicable	04	4.65
Escherichia coli	Negative rod	02	2.33

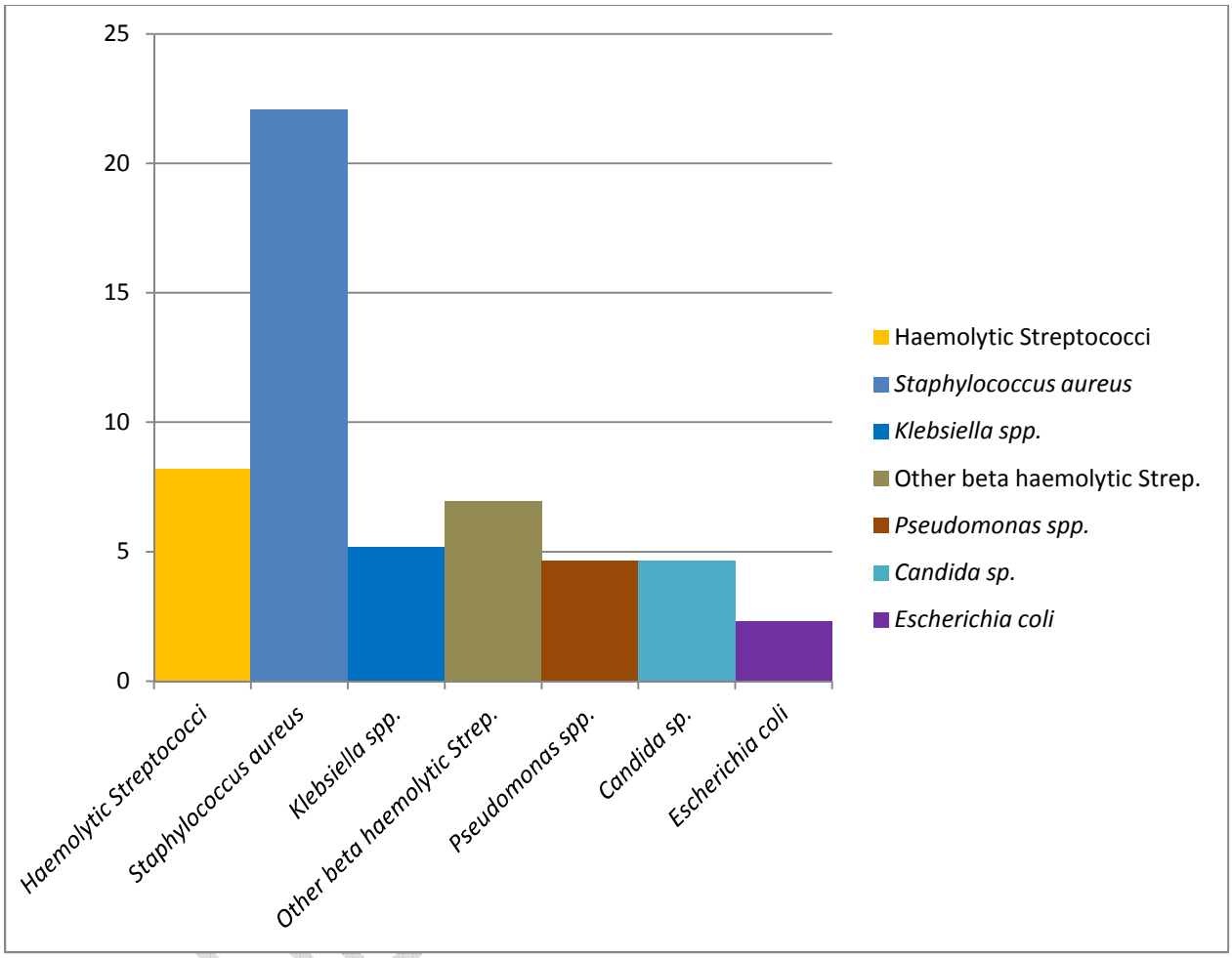

Fig. 1: Histogram Distribution of Other Organisms Isolated from Throat Swab

Other pathogens isolated from the throat swab used in the work include α-haemolytic *Streptococci*(8.2%), *Staphylococcus aureus* (22.1%), *Klebsiella spp* (5.81%), *Pseudomonas spp* (4.65%), *Candida* spp (4.65%), *Escherichia coli* (2.33%). 36 out of 86 samples analyzed showed no growth (41.86%). Others β-haemolytic *Streptococci* isolated was 6.96%.

Discussion

This study found that 5% of throat swab samples examined for detection of Group A *Streptococcus* was identified as *Streptococcus pyogenes.* This is small compared to the report from the study done in Jos which recorded 10.45% [8]. This result is higher than 0.8% rate recorded by Uzodimma *et al.,* from patient between age of 3 – 15 years [9]. Both studies used the same methods which were utilized in this study.

Generally, the prevalence rate of *Streptococcus pyogenes* is low compared to other studies reported in other parts of the country. in 1994, similar study was done in Port Harcourt and out of 116 throat swab analysed, 16 (13.99%) were identified as *Streptococcus pyogenes* by Lurie *et al.,* [10] attributed the high prevalence to poor socioeconomic status, as 94 percent of patients used in the study belonged to low social clauses with an average of four children per family. In addition to this factor, poor health, intelligence in the community accounted for high prevalence rate [10].

Season of the year has been reported as a factor affecting the prevalence rate of *S. pyogenes* [11]. This was also observed in this study as the positive cases recorded were all isolated within same months, a month of constant rain fall.

Streptococcus pyogenes infection is common among children than adults. These authors recorded 15 – 30% cases in children and 5 – 10% cases in adult and it was in concordance with

the result obtained from this study.*Streptococcus pyogenes* is a critical public health issue because its infection often leads to post streptococcal squeal and carrier of this infection serve as source for the spread of infection to other individuals in the community [12].

High prevalence of *Streptococcus pyogenes* has been recorded in some parts of India. In Belgaum city situated in India, 30% prevalence rate was recorded among primary school children [12]. In Chennai also in India, the rate is very high (53.5%) and similar to the result obtained from other parts of the country [6, 13]. In other countries, the prevalence rate vary widely from as low as 9.2% to as high as 28.9% from children [14, 15]. The difference prevalence could be attributed to difference in climatic condition, socio-economic conditions and geographical regions.

Some studies reported higher prevalence in females than in males [16]. This study although very low prevalence positive cases were more of male (2:1) than female the studies that recorded high rate in females reported that it is possible that patients give more attention to boy child than to girl child [16].

Some studies also looked at the socio-economic status of patients involved in the study and recorded that the prevalence was higher among patients from the higher socio-economic group than those from low socio-economic group and attributed it to urbanization and negligence from parents towards children, busy schedule of the family and over confidence in health issues. This present study did not put into consideration the effect of such factors to the prevalence rate [17].

Conclusion

The disease causing potential of *Streptococcus pyogenes* is a public health issue of great concern. Its proper diagnosis and laboratory identification is crucial in management of such diseases. *S. pyrogenes* is a neglected tropical microorganism because of difficulties associated with its identification especially in developing countries. This present study has shown that it exists in the study area but due to improper diagnostic procedures, it was taken that its present in this area is negligible. Hence, care should be taken in processing clinical samples properly for sputum identification of pathogenic organisms. This includes proper sample collection, processing using appropriate media and reagents and good statistical analysis.

REFERENCES

[1] Malota, M., Felbinger, T. W., Rupport, R. & Nisselr, N. C. (2015). Group A *Streptococci* and rare and often misdiagneset cause of spontaneous, bacterial peritonitis in adult. *International Journal of Surgical Cases Reports,* 6, 251 – 255.

[2] Kpipana, S., Sundar, J. J. S., Parameshwari, S., Kaganadum, P., Selevan, J. M., Valamahi, S. & Data, M. (2012). Isolation and identification of group A *Streptococcal* infection among slum chicken in the age group of 5 1- 15 years in Chemni – one year prospective study. *Journal of Pharmacy and Biological Sciences,* 2(1), 27 – 30.

[3] Whan, Z. Z. (2017). Group A *Streptococcus* (GN) infections Articles on infectious diseases. Medscope.com

[4] Beres, S. B., Carroll, R. K., Shea, P. R., Sitkiewiez, I. Martinz, J. C., Law-De, M. A., Willey, R. M., Tyreell, G. J., Goldman, T. D., Feldgarden, M. & Muscer, J. M. (2010). Molecular complexisty of successive bacterial epidemics decontrolled by comparative pathogenomic. *National Academy of Science,* 107, 4371 – 4376.

[5] Fartima, P. & Shuhba, D. S. (2013). Prevalence survey for assessing intensity of group a beta haemolytic *Streptococci* (GABHS) subclinical infection rate in school children: A cross sectional study global. *Journal of Medical Research Diseases,* 13(3), 321 – 328.

[6] Biano, A. L. & Stevens, D. L. (2005). *Streptococcus pyogenes* in Mandell, G. C., Bennett, J. E. and Dolin, R. editors: Principles and practice of infectious diseases, (6th edtion), Philadelphia, Elsevier Churchill, Livingstone.

[7] Courteney, H. S., Ofek, I. & Hasty, D. L. (1997). M protein medicated adhesion of M type 24 *Streptococcus pyogenes* stimulates redegree of interleukin-b by Ht p-2 tissue culture cells. *FEMS Microbiology Letters,* 15(1), 65 – 70.

[8] Walker, M. J., Barnett, T. C., McArthur, J. D., Cole, J. N., Gullen, C. M., Henningham, A., Sripnakash, K. S., Sadersan, M. L. & Nizet, V. (2014). Disease manifestations and pathogenic mechanisms of Group A *Streptococcus.* *Clinical Microbiology Review,* 27(2), 264 – 301.

[9] Uzodinmma, C. C., Dedeke, F. I., Nwadike, V., Owolabi, O., Arifolo, G. & Oduwole, O. (2017). A study of *Streptococcal* pharyngitis among 3 – 15 years old children attending clinics for an acute sore throat. *Nigerian Journal of Cardiology,* 14(2), 94 – 102.

[10] Lurie, S., Vaknine, H., Izakison, A., Levy, I., Sadar, O. & Golden, A. (2017). Group A *Streptococcus* causing a life-threatening postpartum necrotizing myemetitis: A case report. *The Journal of Obstetrics and Gynecology Research,* 34(4), 645 – 648.

[11] Stevens, D. L. (1997). The toxic of group A *Streptococcus,* the flesh eating bacteria. *Immunology Investment,* 26(12), 129 – 150.

[12] Rijal, R. R., Dhakal, N., Shal, R. C., Timilsina, S. & Mahato, P. (2009). Antibiotic susceptibility of group A *Streptococcus* isolated from throat swab culture of school children in Pokhara, Nepal. *Nepal Medical College Journal,* 11(4), 238 – 240.

[13] Chopra, P. & Gulwani, H. (2007). Pathology and pathogenesis of rheumatic heart diseases. *Indian Journal of Pathological Microbiology,* 50(4), 685 – 687.

[14] Cunningham, M. W. (2000). Pathogenesis of group A *Streptococcal* infections. *Clinical Microbiology Review,* 13(3), 470 – 511.

[15] Ekelund, K., Darenberg, J., Norrby-Trylund, A., Hoffmann, S., Bang, D., Skinhoy, P. & Konnadsen, H. B. (2005). Variations in emm type among group A *Streptococcal* isolates causing invasive or non-invasive infections in a nationwide study. *Journal of Clinical Microbiology,* 40, 3101 – 3109.

[16] Henninham, A., Gillen, C. M. & Walker, M. J. (2013). Group A *Streptococcal* vaccine conditions: Potential for the development of a human vaccine. *Current Tropical Microbiology and Immunology,* 368, 207 – 242.

[17] Dhanda, V., Vohra, H. & Kumar, R. (2011). Group A *Streptococcus* virulence factors genes in North India and their association with emm type in pharyngitis. *Indian Journal of medical Research,* 133, 110 – 115.

Bosek, A. L., Wilenska, J., Ledebski, R. & Sitkiewiez, I. (2011). A new rapid and cost effective method for detection of phagis ICEs and virulue factors encoded by *Streptococcus pyogens. Poland Journal of Microbiology,* 60(3), 187 – 201.

Callister, M. E. & Wall, R. A. (2001). Descending necrotizing mediatinitis caused by Group A *Streptococcus* (serotymiti). *Journal of Infectious Diseases,* 33(10), 771 – 772.

Chessbroush, M. (2010). District laboratory practice in tropical country, Part 2, Cambridge University Press.

Chior, C. S., Wang, Y. W., Chen, P. L., Wang, W. L. & Wei, H. L. (2009). Association of the shuffling of *Streptococcus pyogenes* clones and the fluctuation of scalet fever cases between 2000 and 2006 in Central Tawen. *British Medical College of Microbiology,* 9, 115 – 116.

Frner, J. D. & Proft, T. (2008). The bacterial superantigen and superantigen-like proteins. *Immunology Review,* 225, 226 – 243.

Goldberg, G. N., Hansen, R. C. & Lynch, A. J. (1984). Necrotizing fascutis in infancys report or their cases and review of the literature. *Paediatric Dermatology,* 2(1), 55 – 63.

Gunningham, M. W. (2000). Pathogenesis of Group A *Streptococcal* infections. *Clinical Microbiology Review,* 13(3), 470 – 511.

Maltezon, H. C., Isagelis, V., Artonider, A., Golani, L., Dovros, C. & Katsarolis, L. (2008). Evaluation of a rapid antigen detection in the diagnosis of *Streptococcal pharyngitis* in children and its infection antibiotic prescription. *Journal of Antimicrobial Chemotherapy,* 62(6), 1407 – 1412.

Mani, R., Magabise, A., Prathan, S., Nagrathma, S., Srikanth, N. S. & Dias, M. (2007). Fatal Group A *Streptococcus* causing a life-threatening postpartum necrotizing myemetritis in adult. *Indian Journal of Microbiology,* 25(2), 169 – 170.

Mawak, J. D., Ewelike, J. C., Lar, P. M. & Zumbes, H. J. (2006). Bacterial etiologic agents associated with upper respiratory infections in children (under five years) attending selected clinics in Jos, Nigeria. *Highland Medical Research,* 4, 20 – 30.

Metzgar, D. & Zampolli, A. (2011). The N-protein of group A *Streptococcus* is a key virulence factor and a clinically relevant strain identification marker. *Virulence,* 2, 402 – 412.

Owobu, A. C., Sadoh, W. E. & Oviawe, O. (2013). *Streptococcal* throat carriage in a population of nursery and primary school pupils in Benin City, Nigeria. *Niger Journal of Pediatrics,* 40(4), 389 – 394.

Raza, S., Kundu, K. K. & Dutta, S. K. (2013). Prevalence of asymptomatic pharyngeal carriage of β-haemolytid group A *Streptococcus pyogens* among school going children of age 5 – 12 years in Bharapur. *Nepal Journal of Kathmands Medical College,* 2(3), 18 – 20.

Shea, P. R., Ewbank, A. L., Gonzalez-Lugo, J. H., Martagon, A. J., Marharz-Grienez, J. C., Rehman, H. A., Semano-Gonzah, M., Fillpidi, N., eres, S. B., Willey, B. M. & Mosses, J. M. (2011). Group A *Streptococcus* emm gene types in phargngeal isolates, Onteria Canada, 2002 – 2010. *Emerging Infectious Diseases,* 17, 2010 – 2017.

Snider, L. A. & Swedo, S. E. (2003). Post-*Streptococcal* autoimmune disorders of the central nervous system. *Current Opinion in Neurology,* 16(3), 359 – 365.

Steed, A. C., Law, I., Matalolv, L., Beall, B. W. & Carapatis, J. R. (2009). Global emm type distribution of group A *Streptococci*: Systemic review and implications for vaccine development. *Lancet Infectious Diseases,* 9, 611 – 616.

Stevens, D. L. (1995). Streptococcal toxic shock syndrome: Spectrum of diseases, pathogens and new concepts in treatment. *Journal of Emerging Infectious Diseases,* 1(2), 69 – 78.