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Epidemiological Survey of Tonsillitis Caused By *Streptococcuspyogenes*among Children in Awka Metropolis (A Case Study of Hospitals in Awka Community, Anambra State)

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Abstract: *The epidemiological survey of tonsillitis caused by Streptococcus pyogenes among children in Awka metropolis was carried out using samples from three different hospitals. A total number of 73 throat swabs were collected from children aged between 5weeks - 5 years. Swab sticks were streaked onto Blood agar and MacConkey agar for isolation of the test organism. Organisms recovered were Streptococcus pyogenes, Streptococcus pneumoniae, Escherichia coli, Staphylococcus species, Proteus species, Pseudomonas species and Klebsiella species. Streptococcus pyogenespresented the highest number; 19 out of the 39 bacterial isolates recovered. The results showed that the prevalence of bacterial tonsillitis, specifically Streptococcus pyogenes, is 8% in infants aged between 5weeks to 11months and 17% in children aged between 1-5 years. From the results, the higher percentage of the prevalence of bacterial tonsillitis in children aged 1-5 years showed that school children are quite susceptible to Tonsillitis. This is because tonsillitis can be transmitted directly from person to person and indirectly via formites as well as respiratory secretions.This research work has shown that the drugs, Cefuroxime, Azithromycin, Ceftazidine and Gentamicin were 100% sensitive to Streptococcus pyogenes, hence can be recommended for the control of this organism.*

Keywords: *Antibiotics,Bacterial isolates,Streptococcus pyogenes,Throat Swabs,Tonsillitis,*

I. Introduction

Tonsillitis is the inflammation of the tonsils (two oval-shaped pads of tissue at the back of the throat-one tonsil on each side)due to infection. It is mostly caused by viral or bacterial infection [1]. When caused by a bacterium belonging to the Group AStreptococcus, it is typically referred to as 'Strep throat'. The overwhelming majority of people recover completely, with or without medication, in other words, it is a self-limiting disease [1]. In 40%, symptoms will resolve in three days, and within one week in 85% of people, regardless of whether Streptococcal infection is present or not [2]. The tonsils are suspected to help in the immune system to protect the body from infections that may enter the mouth. When these tonsils become infected, they become enlarged and red, and have a yellow or white coating [3]. Tonsil infections may be contagious, and can spread from person to person by contact with the mouth, throat, or mucous of someone who is infected. Generally, tonsillitis symptoms include a sore throat, fever, swollen glands in the neck, and trouble swallowing [4]. The common signs and symptoms include: sore throat, red swollen tonsils, pain when swallowing, high temperature (fever), coughing [3] headache, tiredness, chills, a general sense of feeling unwell (malaise), white pus -filled spots on the tonsils, swollen lymph nodes (glands) in the neck, pain in the ears or neck, weight loss, difficulty ingesting and swallowing meal/liquid intake while the less common symptoms include: nausea, fatigue, stomach ache, vomiting, furry tongue, bad breath (halitosis), voice changes, difficulty opening the mouth (trismus), loss of appetite (anorexia), anxiety/fear of choking [1]. In cases of acute tonsillitis, the surface of the tonsil may be bright red and with visible white areas or streaks of pus [5]. Tonsilloliths occur in up to 10% of the population frequently due to episodes of tonsillitis [6]. The most common cause is viral infection and includes adenovirus, rhinovirus, influenza, coronavirus, and respiratory syncytial virus [7].The second most common cause is bacterial infection of which the predominant is Group A β-hemolytic Streptococcus (GABHS), which causes strep throat. Less common bacterial causes include: *Staphylococcus aureus* (including methicillin resistant *Staphylococcus aureus* or MRSA), *Streptococcus pneumoniae*, *Mycoplasma pneumoniae*, *Chlamydia pneumoniae*, *Bordetella pertussis*, *Fusobacterium species*, *Corynebacteriumdiphtheriae*, *Treponemapallidum* and *Neisseria gonorrhoeae* [1; 8]. Anaerobic bacteria have been implicated in tonsillitis and a possible role in the acute inflammatory process is supported by several clinical and scientific observations [7]. Under normal circumstances, as viruses and bacteria enter the body through the nose and mouth, they are filtered in the tonsils [9]. Within the tonsils, white blood cells of the immune system destroy the viruses or bacteria by producing

inflammatory cytokines like Phospholipase A2 [10]which also lead to fever [9;11]. The infection may also be present in the throat and surrounding areas, causing inflammation of the pharynx [5].

Streptococcus pyogenes (group A streptococcus) is a Gram-positive,non-spore forming cocci arranged in chains or pairs which causes some of the most common infections in humans [12]. *Streptococcus pyogenes* causes pyogenic infections, with a characteristic tendency to spread, as opposed to Staphylococcal lesions which are typically localized. It is also responsible for the non-suppurative lesions, Acute Rheumatic Fever and Glomerulonephritis, which occur as sequeal to *Streptococcuspyogenes* infection [13]. The metabolism of *Streptococcus pyogenes*is fermentative; the organism is a catalase-negative aerotolerant anaerobe (facultative anaerobe), and requires enriched medium containing blood to grow [14].The two most common infections caused by *Streptococcus pyogenes*are acute tonsillitis ('Strep throat') and the skin infection impetigo. Rheumatic Fever mainly occurs in 1-3% of streptococcal throat infection of children living in poor condition. Acute tonsillitis is most commonly seen in cold seasons and early in temperate climates, although it may occur at any time of the year. The rates of isolation and spread of group A streptococcal infection in closed and crowded communities may be much higher. Throat culture has always been considered the "gold standard" for diagnosing group A streptococci. The incubation period for streptococcal tonsillitis is short (2 to 5 days). Researches pertinent to group A Streptococcal infections are scarcely available in Nigeria [14]. No such community based study has been conducted. Only some hospital based study has been done. It has been shown that the incidence of the Streptococcal infection is higher among the less than 15 years population with poor socio-economic status. Hence, this study was done to know the prevalence of the group A streptococcal infection (tonsillitis) which will be useful for the programmers' planning and to control group A streptococcal infections.

II. Materials And Methods

Children presenting with tonsillitis at the Regina Caeli Hospital, Izunna Hospital, and Beacon Hospital, Awka,Anambra State were recruited for the study.

Inclusion criteria: Patients with a history of sore throat, difficulty in swallowing, and fever (including a recorded temperature of $>38^0$C at presentation) and/ or evidence of inflamed tonsils.

2.1 Collection and Characterization of Isolates

Throat swabs were collected by the attending pediatrician from each patient using a sterile swab stick. The swab stick was introduced into a well exposed mouth with the tongue depressed by a wooden spatula where necessary and visible exudates or hyperemic areas on the tonsillar walls were swabbed. The swab stick was carefully replaced in its sheath and sent for Microbiological analysis within 1 hour of collection. Swabs were simultaneously plated on a Blood agar and MacConkey media and incubated at 37^o C for 48 hours. Growths were further sub-cultured to identify species.

2.2 Identification of Isolates:

Colony morphology was done to identify the size, colour, as well as surface features of the colonies usingstandard procedures as described by Cheesbrough [15].SelectedBiochemical tests used to identify the test organisms were also carried out in reference to Cheesbrough [15]. The isolated organisms were also identified using Gram staining reaction and Bacitracin Susceptibility disk test.

2.3 Antibiotic Susceptibility Test:

Antibiotic Susceptibility test was done using Disk Diffusion method.A small portion of the test organism was streaked on Mueller-Hinton agar plate using a sterile wire loop as explained by Ogbo [16]. The Antibiotic test rings were asceptically picked and placed on the agar plates using a sterilized mounting forceps. The plates were finally closed and incubated for 24hrs at 37^0C.

2.4 Statistical Analysis

Data were analyzed according to Freedman [17] and simple proportions were represented in percentages. The sensitivity (the number of patients with bacteria isolates as a percentage of patients with the symptom) and specificity (the number of patients without bacterial isolates as a percentage of patients without the symptom) of each of the clinical symptom/sign in predicting possible bacterial tonsillitis were determined. The mean age of those with isolates was 2.30years (range, 5weeks-5years) and it was determined using the Z-test. Chi-square was used to test associations based on sex, age distribution, and prevalence of tonsillitis caused by *Streptococcus pyogenes*as compared to other organisms.

III. Results

There were 73 patients who met the study criteria and their samples were analyzed. Of which 41(56.16%) were females and 32(43.84%) were males, giving an M: F ratio of 1:1.3. The mean age was 1.97years (approximately 2years). 19(26.03%) were infants and 54(73.97%) were aged between 1 and 5 years. Most of the patients 54(73.97%) had tonsillitis, 11(15.07%) had tonsillopharyngitis while 8(10.96%) had pharyngitis.

Fever and inflamed tonsils were the most frequent signs or symptoms occurring in 65 (89.04%) and 64 (87.67%) of subjects respectively while cervical adenitis 20 (27.39%) and presence of exudates 19 (26.03%) were the least frequent signs (Table 1). Although all the clinical features showed relatively low sensitivity and specificity, inflamed tonsils (64.81) and cough (64.28%) were the most sensitive while fever (55.38%) and cervical adenitis (55.00%) were the least sensitive. Cough (54.84%) and presence of exudates (50.00%) showed the most specificity while fever (37.50%) and inflamed tonsils (15.80%) had the least specificity.

The most frequently associated disease was Malaria 17 (23.29%) and Otitis media 4 (5.48%). Bronchial asthma and Paediatric AIDS were present in 3 (4.11%) patients each. Others were Measles, Mumps, Rheumatic heart disease and Sickle cell anaemia which had occurred in 1 (1.37%) patient each.

Growths of *Streptococcus pyogenes*, *Streptococcus pneumoniae*, *Staphylococcus species*, *Klebsiella species, Pseudomonas species*, *Proteus species* and *Escherichia coli* were identified using enriched and differential media (Blood agar and MacConkey agar), Gram stain technique, bacitracin susceptibility test as well as biochemical tests (catalase test, oxidase test, bile solubility test) (Table 2);

Using Blood agar and MacConkey agar; *Streptococcus pyogenes* exhibited a clear and complete haemolysis on blood agar while *Streptococcus pneumonia* appeared as a mucoid stain on the blood agar appearing as a central depression, indicating alpha haemolysis. On MacConkey agar, colonies of *Escherichia coli* were seen as non-mucoid pink colonies, those of *Klebsiella species* were seen as large, pink and mucoid colonies, Staphylococcus species were seen as pale pink colonies, *and Proteus species* was seen as colourless colonies while *Pseudomonas* was seen as large green-brown colonies.

After isolation of the organisms from the different agar used, the organisms were Gram stained and *Streptococcus pyogenes* and *Streptococcus pneumoniae* were seen as Gram positive cocci some appearing in chains and others in pairs, *Staphylococcus* species was also seen as Gram positive cocci but appearing in clusters. *Escherichia coli*, *Klebsiella species*, Proteus species and Pseudomonas species were seen as Gram negative rods.

The catalase test done indicated that *Streptococcus pyogenes* had a negative catalase activity, while *E. coli, Klebsiella species, Pseudomonas species*, and *Proteus species* had positive catalase activity.

Streptococcus pyogenes was further identified and confirmed using the Bacitracin susceptibility disk (A disk) where it exhibited a small zone of inhibition around the disk.

In summary, 7 different bacterial isolates were isolated from 39 (53.42%) subjects. 19 (48.72%) of the isolates were *Streptococcus pyogenes*, while 5(12.83%) were *Staphyloccocus species*. Klebsiella species, *Streptococcus pneumoniae*, *Proteus species*, *Pseudomonas species* and *Escherichia coli* were recovered from the throat of 5 (12.83%), 3 (7.69%), 3 (7.69%), 3(7.69%) and 1(5.13%) subjects respectively. Of the 39 subjects with isolates, 17(43.59%) were males and 22 females giving M: F ratio of 1:1.3.

The sensitivity of the isolates was tested against nine common antibiotics, using Biotec Gram positive and Gram negative disks (Batch number C124679) with disk diffusion method.

*Streptococcus pyogenes*and *Staphylococcus species* from the different cases, all showed 100% sensitivity to cefuroxime, azithromycin, ceftazidine and Gentamicin but showed little or no sensitivity to ampicillin and co-trimoxazole, (Table 3).

TABLE 1: The sensitivity, specificity of clinical features used in predicting tonsillitis.

Symptoms/Signs	No with Symptoms (%)	No without Symptoms	No with Isolates	No without isolate	Sensitivity %	Specificity %
Fever	65 (89.04)	8	36	3	55.38	37.50
Cough	42 (57.53)	31	27	17	64.28	54.84
Sore throat	20(27.39)	53	12	25	60.00	47.17
Difficult swallowing	20 (27.39)	53	12	23	60.00	47.17
Inflamed tonsils	54 (73.97)	19	35	3	64.81	15.80
Exudates	19 (26.03)	54	12	27	63.16	50.00
Cervical adenitis	20 (27.39)	54	11	26	55.00	48.15

No= number

TABLE 2:Summary of the identification of isolated organisms

Organism	**Appearance on the Agar**		**Gram reaction**	**Catalase test**	**Oxidase test**	**Bacitracin test**	**Bile solubility**
	BA	**MA**					

							test
Strept.pyogenes	Bhaemolysis		+	-		Sensitive	
Strept.Pneumonia	Ahaemolysis		+	-			Soluble
Staphylococcus Species		Pale pink	+	+			
Klebsiella species		Pink, mucoid	-	+			
Proteus species		Colourless	-	+	+		
Escherichia coli		Pink, non-mucoid	-	+			
Pseudomonas species		Greenish – brown	-	+			

TABLE 3: Antibiotic sensitivity of bacterial isolates

Antibiotics	*Streptococcus pyogenes*	Staphylococcus Species	Klebsiella Species	Pseudomonas species	Proteus species
Cefuroxime	100	100	100	66.7	100
Gentamicin	100	100	83.3	100	100
Azithromycin	100	100	100	100	100
Ceftazidine	100	100	83.3	100	66.7
Amoxicillin	88.2	100	50	0.0	100
Streptomycin	80.0	100	33.3	33.3	100
Ampicillin	64.7	75	50	33.3	100
Floxapen	58.8	75	100	33.3	66.7
Cotrimoxazole	11.1	25	0.0	0.0	66.7

Key: Percentage (%) of Isolates sensitive toAntibiotics

IV. Discussion

It has been established that tonsillitis caused by *Streptococcus pyogenes* occur more in children than in adults [18]. The fact that these young children are still in the process of developing immunity to pathogens in their environment may explain these findings. Although sore throat and difficulty in swallowing are major symptoms in tonsillitis[19], less than a third of the patients in this study presented with these complaints. This is because children seldom complain of sore throat or difficulty in swallowing. Since almost a third of the studied patients were infants, complaint of sore throat or difficulty with swallowing would obviously be less.

The use of individual clinical features as predictors of possible bacteria tonsillitis was poor as shown by their low sensitivity and specificity. This is consistent with other studies that showed the unreliability of using individual sign to predict possible bacterial tonsillitis [20]. Almost half of the bacteria isolates were *Streptococcus pyogenes*, the predominance of this organism in this study is consistent with reports of other studies [19]. The unusual presence of Gram negative coliform organisms (Klebsiella, Proteus and Pseudomonas) in this study is noted. Since they are not traditional upper respiratory pathogens, it is possible that they could have been transferred from the anal region to the throat following poor personal hygiene [7]. The fact that the patients with coliform organisms were significantly younger lends credence to such possibility.

Our study showed that *Streptococcus pyogenes*and Staphylococcus species had 100% sensitivity to Cefuroxine, Azithromycin and Ceftazidime, three relatively expensive drugs, a finding corroborated by previous studies [20;21]. It is worthy of note that Amoxicillin showed more activity against the pathogens isolated compared to Ampicillin in this study, despite the fact that both drugs are amino-penicillins and are supposed to have similar antibacterial activities [22]. Although the reason for this difference is not clear, it is suspected it may be related to possible abuse of the cheaper ampicillin with resultant resistance.

The coliform organisms showed good sensitivity to Gentamicin, an affordable drug. Gentamicin would be particularly useful where the parenteral route of drug administration is indicated. However its suitability as a first line drug in infections by these pathogens is limited by the fact that it can only be administered parenterally[23].

V. Conclusion And Recommendation

No death was recorded following the results gotten. But a possibility of death can occur in infants especially the immunocompromised as they are susceptible to complicated diseases from Tonsillitis. Females had higher disease risk although this is not statistically significant as from the analysis; gender had no influence on the number. School children are quite susceptible to the disease because tonsillitis can be transmitted directly from person to person as well as via formites.

Analysis of the project data also brought about some new insights into the possible risk factors as well as possible predictors of the disease. This has in turn allowed an evaluation of risk to contacts, forming the basis of guidance on the management of cases of *Streptococcus pyogenes*infection arising in the community to help better understand the need for the diagnosis of tonsillitis. Although this study was about the survey of tonsillitis

caused by *Streptococcus pyogenes*, tonsillitis can as well be caused by many other bacteria, viruses as well as environmental factors.

From the study, it was observed that people generally take tonsillitis as a self-limiting disease without knowing that severe damages could arise from *Streptococcuspyogenes* infections if not well treated. From this study, it is recommended that once the signs and symptoms are observed even in the earliest stage, proper medical measures should be taken to enable better diagnosis and treatment by a qualified medical practitioner. Self- medication should be seriously discouraged to reduce the risk of resistance by organisms.

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