

Bacterial Tonsillitis and Antimicrobial Resistance Profiles Among Children Within Five Years of Age At Hargeisa Group of Hospital, Somaliland: A Cross-Sectional Study

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Research Article

Keywords: Children, tonsillitis, bacteria, antimicrobial resistance, Hargeisa group Hospital

Posted Date: June 12th, 2021

DOI: <https://doi.org/10.21203/rs.3.rs-586545/v1>

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Abstract

Background

Tonsillitis is the third most frequently diagnosed infection in the pediatrics age group around the world. It causes significant morbidity and loss of school attendance in children. The emergence of drug resistance in bacterial tonsillitis is getting higher every year. However, data on the drug resistance profiles of bacterial causes of tonsillitis among children within five years of age is not available in Somaliland. Therefore; this study determined the bacterial causes of tonsillitis and their antimicrobial resistance profiles among children within five years of age at Hargeisa Group of Hospital, Somaliland.

Methods

A cross-sectional study was conducted from March to July 2020. A total of 374 children within five years of age were included using convenient sampling method. Throat swabs were collected from children, processed and bacterial species were identified using standard bacteriological procedures. Antimicrobial susceptibility was done using disc diffusion method. Data on demographic variables and clinical profiles were collected using structured questionnaires. Logistic regression analysis was computed to identify factors associated with bacterial tonsillitis. P-values < 0.05 were taken as statistically significant.

Results

The median age of children included in the study was 4 years. Overall, 120(32.1%)(95% CI 27.4–36.8%) of children had culture confirmed bacterial tonsillitis. Of them, 23(19.2%) had mixed infections. The most frequent bacterial isolates were *Streptococcus pyogenes* 78(55%), *Staphylococcus aureus* 42 (29%) and *Streptococcus pneumoniae* 10(7%). Isolates revealed 83.3–100 % rate of resistance to ampicillin. *S. aureus* was resistant to clarithromycin (38%) while 60% of *S. pneumoniae* isolates were resistant to gentamicin. The overall multidrug resistance (MDR) was 50.4% and 52.6% of *S. pyogenes* and 60% of *S. pneumoniae* were MDR. History of tonsillitis (AOR = 0.12; 95% CI = 0.06–0.21), difficulty of swallowing (AOR = 6.99; 95% CI = 3.56–13.73), weight loss (AOR = 0.33; 95% CI = 0.186–0.597) and attending school (AOR = 2.98; 95% CI = 1.64–5.42) were found to be associated with tonsillitis among children within five years of age.

Conclusions

Bacterial tonsillitis with high degree of ampicillin resistance, mixed infections and MDR isolates are major concerns in children within five years of age at Hargeisa, Somaliland. Therefore, treatment of cases should be guided by regular culture and antimicrobial susceptibility testing to prevent disease consequences and critical drug resistance.

Background

Tonsillitis is the inflammation of tonsils. It is contagious, and can spread through close contact with infected persons, sharing food, drinks and utensils. Tonsillitis impacts the health of children, their quality of life and causes significant morbidity and loss of time for schooling [1]. Poor living conditions, exposure to environmental pollutants and indoor air pollution are frequently reported factors associated with tonsillitis among children within five years of age [2].

Gram positive and Gram negative bacteria are frequently associated with symptomatic childhood tonsillitis. Of them, *Streptococcus pyogenes* (*S. pyogenes*), methicillin resistant *Staphylococcus aureus* (MRSA), *Streptococcus pneumoniae* (*S. pneumoniae*), *Haemophilus influenzae* (*H. influenzae*), *Moraxella catarrhalis* (*M. catarrhalis*), *Pseudomonas aeruginosa* (*P. aeruginosa*) and *Klebsiella pneumoniae* (*K. pneumoniae*) are the common etiologies of tonsillitis [3].

The emergence of drug resistant bacteria in tonsillitis is getting higher every year. Methicillin resistant *S. aureus* (MRSA) is one of the most frequent bacterial causes of tonsillitis in children. Irrational use of antibiotics by patients, production of beta-lactamase enzymes and formation of biofilms are the main reasons for emergence of drug resistance [4]. The spread of drug resistant bacteria has led to treatment failure and recurrence of tonsillitis among children with poor sanitation and hygiene in underdeveloped countries.

The situation is critical in Somaliland, where antimicrobials are vastly and frequently used irrationally. This might increase the emergence of resistance to the commonly used antibiotics for the treatment of tonsillitis [5].

In Hargeisa Group of Hospitals, like other similar health settings in Somaliland, routine culture and antibiotic susceptibility testing are not usually performed as essential part of patient care and treatments are mostly based on empirical therapy. However, there are no studies conducted and published on the culture confirmed burden of bacterial tonsillitis and antimicrobial resistance profiles as well as the extent of MRSA in children within five years of age in Somaliland. Moreover, asymptomatic children can be the sources of dissemination of bacteria causing tonsillitis to non-infected children at home or at school settings that can lead to wide range of tonsillar infections. Therefore, we present the first report of the prevalence of bacterial causes of tonsillitis and antimicrobial resistance profiles of the isolates in children within five years of age at Hargeisa Group of Hospital, Somaliland.

Methods

Study design, period and setting

A hospital based cross-sectional study was conducted between March and July 2020 in Hargeisa Group of Hospital (HGH), Somaliland. Hargeisa Group of Hospital is located in Maroodi Jeex Region, the capital city of Somaliland known as Hargeisa. According to the 2019 census report from Central Statistics Department of Somaliland, Hargeisa has a total population of 1.2 million. Hargeisa Group of Hospital is the largest referral public hospital with more than 200 healthcare professionals. It is one of the health hubs in Somaliland. The pediatric department of HGH has outpatient and inpatient departments with 15 Pediatricians and two Nurses. Daily 50 outpatients and 1 to 4 hospitalized children attend the Pediatric Clinic for different medical conditions. All children within five years of age with tonsillitis at Ear, Nose and Throat (ENT) of HGH were the study population.

Inclusion and Exclusion Criteria

Children within five years of age presumptive for tonsillitis with sore throat, red swollen tonsils, pain when swallowing, fever, cough, tiredness, malaise, and white pus-filled spots on the tonsils, swollen lymph nodes, pain in the ears or neck, and weight loss were included in the study. On the other hand, children who were on antibiotic treatment within the previous two weeks of sample collection, and had tonsillectomy were excluded from the study

Sample size and Sampling

The sample size was calculated using single population formula $n = (Z\alpha/2)^2 P (1-P)/d^2$ where, n = sample size, Z = level of confidence according to the standard normal distribution, P = sample proportion and d = tolerated margin of error. Therefore; by taking $Z(\alpha/2) = 1.96$ for a level of confidence of 95 %, $P = 0.5$ which is the maximum proportion of bacterial tonsillitis and 5% margin of error, the sample size was calculated as $n = (1.96)^2 \times 0.5 (1-0.5)/(0.05)^2 = 384$. However, due to the lack of sufficient throat swabs and incomplete questionnaire based data, only 374 children within five years of age with tonsillitis were included in the study. Study participants were included conveniently. All children within five years of age with tonsillitis attending at ENT department of HGH and who fulfilled the inclusion criteria were included consecutively until the required sample size was reached.

Variables

Bacterial causes of tonsillitis was the dependent variable while demographic variables (child's age, mother's age, father's age, gender, residence, maternal education, paternal education, and parental occupation), clinical related variables (history of tonsillitis, current type of tonsillitis, number of previous tonsillitis, body temperature, sore throat, swollen tonsils, headache, swollen lymph nodes, difficulty in swallowing, white exudates on the throat, weight loss, tonsillar structural change and history of drug use) were the independent variables. Moreover, patient related variables such as history of contact with someone who had cough, type of breast feeding, attending day care and school, living in overcrowded environment and exposure to wood biofuel were taken as the independent variables.

Data Collection

A structured questionnaire was used to collect data on demographic characteristics, clinical profiles and other variables. Demographic and other related profiles of the children were collected with face - to - face interviews of their caregivers. Clinical data of children with tonsillitis were screened by the attending pediatricians.

Throat Swab Sample Collection and processing

Throat swabs were taken by the attending pediatricians from each patient using a sterile cotton swab. Visible exudates or hyperemic areas on tonsillar walls were swabbed with a sterile cotton swab while the tongue depressed by a wooden spatula when necessary.

All the swab samples were immediately transported to the Microbiology Department of HGH using Amie's transport medium (Oxoid, England). Swabs were simultaneously plated onto Blood Agar (BA), Chocolate Agar (CA), and MacConkey (MAC) Agar and incubated for 48 h at 37°C. Chocolate Agar was incubated in a candle jar to get 5% CO₂ while BA and MAC were incubated at a normal atmosphere.

Identification of bacterial isolates

Pure colonies of the bacterial isolates were identified in to species following standard enzymatic and biochemical tests [6]. Small colonies, Gram positive cocci arranged in chain, forming complete hemolysis on BA and both coagulase and catalase negative were taken as *S. pyogenes* isolates. *S. pneumoniae* isolates were identified by Gram positive alpha-hemolytic small colonies on BA and were susceptible to optochin. *S. aureus* isolates were identified by Gram positive cluster forming glistening golden yellow colonies on BA and Mannitol Salt Agar (MSA) which were coagulase, catalase and oxidase positive. *Moraxella catarrhalis* were identified by large kidney shaped diplococci Gram negative grey to white hemispheric colonies on BA with both oxidase and catalase positive. *K. pneumoniae* and *P. aeruginosa* isolates were identified by standard manual biochemical tests.

Antimicrobial susceptibility testing

Susceptibilities of all the identified bacterial isolates to different antimicrobials were performed according to the criteria of Clinical and Laboratory Standards Institute (CLSI, 2019) using the Kirby-Bauer disc diffusion method on Mueller-Hinton Agar (MHA) (Himedia, India). The following drug discs were tested: ampicillin (10 µg), amoxicillin-clavulanic acid (20/10 µg), gentamicin (10 µg), clarithromycin (15 µg), erythromycin (15 µg), vancomycin (30 µg), ofloxacin (5 µg) and ciprofloxacin (5 µg). A loop full of culture was taken from a pure culture colony and transferred to a tube containing 5 ml of normal saline and mixed gently until it forms a homogenous suspension. The turbidity of the suspension was then adjusted to the turbidity of McFarland 0.5 (which carries 10⁸ CFU/ml) and was swabbed on a dry surface of MHA plate (150 mm) using a sterile cotton swab. Antibiotic discs were dispensed using a single disc dispenser. Plates were then incubated for 24 h at 37°C. Diameters of the zone of inhibition around the discs were measured using a digital caliper. The results of the zone of antibiotics were interpreted based on the 2019 CLSI guideline [7]. These antimicrobial drug discs were selected based on the frequent prescriptions of these drugs for the treatment of tonsillitis infection in the study area and using the 2019 CLSI [7].

Detection of Methicillin Resistant *Staphylococcus aureus*

Methicillin resistant *Staphylococcus aureus* was screened using oxacillin disk diffusion susceptibility testing. Pure colonies of *Staphylococcus aureus* were inoculated on MSA and 30 µg of oxacillin discs were impregnated on the plate and incubated for 18 h. The zone of inhibition was measured by caliper. Measurements from the CLSI 2019 standard was followed as reference for interpretation. Accordingly, a zone of inhibition of ≤ 21 mm of oxacillin disk against *Staphylococcus aureus* isolates were considered as mecA positive and reported as methicillin resistant while if the zone of inhibition of oxacillin disc towards *Staphylococcus aureus* is ≥ 25mm, it was considered as mecA negative, and reported as methicillin sensitive[7].

Quality Control

Specimens were collected properly following standard bacteriological procedures. In order to prevent contamination, all the throat swab specimens were analyzed within two hours of collection. Culture media were checked for sterility. The media were tested every time after preparation for sterility checking by incubating a plate of each medium overnight in a different incubator than one used for culture. The performance of all the prepared culture media were checked by using American Type Culture Collection (ATCC) standard reference strains (*S. aureus* ATCC 29213, *S. pneumoniae* ATCC 49618, and *P. aeruginosa* ATCC 27853).

Data analysis

Data were coded and analyzed using IBM SPSS statistics for windows version 25 (IBM Corp, Armonk, NY, USA). Univariate analysis was made to generate summary values for the most important variables. Logistic regression analysis was made to determine the association between dependent and independent variables. The generated data were compiled with frequency tables and other

statistical summary measures. Stepwise logistic regression model was used to find factors associated with culture positive bacterial tonsillitis and statistical significance was set at $p < 0.05$.

Ethical Considerations

An ethical approval letter was obtained from the Institutional Review Board (IRB) of College of Medicine and Health Science (CMHS), Bahir Dar University. A permission letter was obtained from the Ministry of Health, Somaliland, and Hargeisa Group of Hospital (HGH). Following well-versed about the purpose and importance of the study, written informed consent was obtained from children parents/guardians before collecting data. Information obtained during this study was kept confidential and used only for the study purpose. Bacteriological positive results were submitted to health pediatricians.

Results

Characteristics of the study participants

A total of 374 children within five years of age with tonsillitis took part with a response rate of 97.4%. Among them, 200 (53.5%) were males. Most (81.6%) of the children were urban dwellers. The age range of children was 2 to 5 years. Majority (3.7%) of the children were five years old (mean = 4.1, median = 4). The children mother's age ranged from 20–45 years. Most (69%) of the parents were employee (Table 1).

Table 1

Socio-demographic characteristics and culture confirmed bacterial tonsillitis among children within five years of age at Hargeisa Group of Hospital

Variables	Total N (%)	Culture results		COR (95% CI)	P-value
		Positive N (%)	Negative N (%)		
Children age (years)					0.199
2	2 (0.5)	2 (100)	0	NA	
3	110 (29.4)	36 (32.7)	74 (67.3)		
4	121 (32.4)	40 (33.1)	81 (66.9)		
5	141 (37.7)	42 (29.8)	99 (70.2)		
Gender					
Male	200 (53.5)	73 (36.5)	127 (63.5)	0.64 (0.41-1.00)	0.05
Female	174 (46.5)	47 (27)	127 (73)		
Residence					
Rural	69 (18.4)	19 (27.5)	50 (72.5)		0.37
Urban	305 (81.6)	101 (33.1)	204 (66.9)	0.78 (0.43–1.37)	
Father's education					
Not able to read and write	55 (14.7)	18 (32.7)	37 (67.3)	3.46 (0.67 = 17.72)	0.002
Able to read and write	56 (15)	29 (51.8)	27 (48.2)	2.53 (0.46–13.79)	
Primary school	81 (21.7)	19 (23.5)	62 (76.5)	1.01 (0.20–5.57)	
High school	82 (21.9)	30 (36.6)	52 (63.4)	0.9 (0.16–5.04)	
Higher education	100 (26.7)	24 (24)	76 (76)		
Mother's education					
Not able to read and write	186 (49.7)	78 (41.9)	108 (58.1)	NA	0.001
Able to read and write	105 (28.1)	28 (26.7)	77 (73.3)		
Primary school	36 (9.6)	7 (19.4)	29 (80.6)		
High school	30 (8)	6 (20)	24 (80)		
Higher education	17 (4.5)	1 (5.9)	16 (94.1)		
Parental occupation					
Unemployed	101 (31)	26 (22.4)	90 (77.6)	1.98 (1.19–3.29)	0.020
Employed	258 (69)	94 (36.4)	164 (63.6)		
Mother's age (years)					
20–25	98 (26.2)	26 (26.5)	72 (73.5)	NA	0.14
26–30	169 (45.2)	59 (34.9)	110 (65.1)		
31–35	90 (24.1)	27 (30)	63 (70)		
36–40	15 (4)	6 (40)	9 (60)		

Key: NA: Not applicable

Variables	Total N (%)	Culture results		COR (95% CI)	P- value
		Positive	Negative		
		N (%)	N (%)		
41–45	2 (0.5)	2	0		
Total	374 (100)	120 (32.1)	254 (77.9)		
Key: NA: Not applicable					

Bacterial tonsillitis

Overall, 120 (32.1%) of the children had culture confirmed bacterial tonsillitis. The proportion of bacterial tonsillitis was higher in males 76 (36.5%) than females 47 (27%). It was higher in urban 101 (33.1%) than rural 19 (27.5%) residents. The percentage of bacterial tonsillitis was higher (41.9%) in children from mother's unable to read and write than other groups (5.9–26.7%). Children from fathers who had higher educational attainment had lowest percentage of bacterial tonsillitis compared to others (Table 1).

Bacterial tonsillitis and clinical profiles

Table 2 depicts the bacterial tonsillitis with clinical profiles among children within five years of age. From the total, 172 (46%) of children had a history of tonsillitis. The majority of children had presented with acute tonsillitis (54%) and sore throat (91.7%). Swollen tonsils were presented in 98.1% of children. On the other hand, cervical lymphadenopathy was presented in 151 (40.4%) of children. Moreover, 147 (39.3%) and 69 (18.4%) of children had difficulty of swallowing and white exudates, respectively (Table 2).

Table 2
Bacterial tonsillitis and clinical profiles of children with tonsillitis at Hargeisa Group of Hospital

Variables	Culture results			COR (95% CI) P- value	
	Total N (%)	Positive N (%)	Negative N (%)		
History of tonsillitis					
Yes	172 (46)	95 (55.2)	77 (44.8)	8.72 (5.22–14.63)	< 0.001
No	202 (54)	25 (12.4)	177 (87.6)		
Number of previous tonsillitis					
None	202 (54)	26 (12.9)	176 (87.1)		
One	15 (4)	9 (60)	6 (40)	8 (4.63–13.82)	< 0.001
Two	24 (6.4)	14 (58.3)	10 (41.7)	1.38 (0.44–4.35)	0.58
Three	13 (3.5)	6 (46.2)	7 (58.3)	0.84 (0.35–2.1)	0.71
≥ Four	120 (32.1)	65 (54.2)	55 (45.8)	0.79 (0.26–2.35)	0.67
Type of tonsillitis					
Acute	202 (54)	25 (12.4)	177 (87.6)		
Chronic	52 (13.9)	30 (57.7)	22 (42.3)	0.11 (0.68 – 0.19)	< 0.001
Recurrent	120 (32.1)	65 (54.2)	55 (45.8)		
Weight loss					
Yes	143 (38.2)	67 (46.9)	76 (53.1)	2.96 (1.89–4.64)	< 0.001
No	231 (61.8)	53 (22.9)	178 (77.1)		
Tonsillar structural change					
Yes	52 (13.9)	30 (57.7)	22 (42.3)	3.52 (1.93–6.42)	< 0.001
No	322 (86.1)	90 (28)	232 (72)		
Body temperature					
37 C °	42 (11.2)	12 (28.6)	30 (71.4)	1.21 (0.59-)	0.61
> 38°C	301 (88.8)	108 (32.5)	224 (67.5)	1.21(0.59-)	
Sore throat					
Yes	343 (91.7)	111 (32.4)	232 (63.6)	0.86 (0.38–1.92)	0.704
No	31 (8.3)	9 (29)	22 (71)		
Swollen tonsils					
Yes	367 (98.1)	119 (32.4)	248 (67.6)	0.35 (0.04–2.92)	0.308
No	7 (1.9)	1 (14.3)	6 (85.7)		
Headache					
Yes	105 (28.1)	33 (31.4)	72 (68.6)	1.04 (0.64–1.69)	0.865
No	269 (71.9)	87 (32.3)	182 (67.7)		
Swollen lymph nodes					

Variables	Culture results			COR (95% CI) P- value	
	Total N (%)	Positive N (%)	Negative N (%)		
Yes	151 (40.4)	53 (35.1)	98 (64.9)	1.26 (0.81–1.96)	0.304
No	223 (59.6)	67 (30)	156 (70)		
White exudates					
Yes	69 (18.4)	19 (27.5)	50 (72.5)	0.77 (0.43–1.37)	0.370
No	305 (81.6)	101 (33.1)	204 (66.9)		
Difficulty of swallowing					
Yes	147 (39.3)	27 (18.4)	120(81.6)	0.33 (0.19–0.53)	< 0.001
No	227 (60.7)	93 (41)	134 (59)		

The percentage of bacterial tonsillitis was higher among children with a history of tonsillitis (55.2%) than others (12.4%). The percentage of tonsillitis was the highest (55.7%) in children with symptoms of chronic tonsillitis. Moreover, the percentage of bacterial tonsillitis was higher in children with tonsillar structural change (57.7%) than those without (28%). Children with swollen tonsils had higher percentage of culture confirmed tonsillitis (32.4%) than those without swollen tonsils (14.3%). The proportion of culture confirmed bacterial tonsillitis was higher among children who had weight loss (46.9%) than the counters (22.9%) (Table 2).

Bacterial tonsillitis in relation to other variables

Overall, 96 (25.7%) and 228 (61%) of children were exclusively breastfed and had history of contact with coughing patients, respectively. On the other hand, 86 (23%) and 282 (75.4%) of children were daycare center attendees and school attendees, respectively. Most of the children lived in a crowded house (71.7%) and 88.5% had exposure to biofuel (Table 3).

Table 3
Distribution of bacterial tonsillitis and other explanatory variables of children within five years of age with symptoms of tonsillitis at Hargeisa Group of hospital.

Variables	Culture result			COR (95% CI)	P-value
	Total N (%)	Positive N (%)	Negative N (%)		
Contact with cough patients					
Yes	228 (61)	85 (37.3)	143 (62.7)	1.89 (1.18–3.00)	0.008
No	146 (39)	35 (24)	111 (76)		
Breast feeding					
Mixed	278 (74.3)	91 (32.7)	187 (67.3)	1.12 (0.68–1.86)	0.65
Exclusive	96 (25.7)	29 (30.2)	67 (69.8)		
Day care center attendee					
Yes	86 (23)	34 (39.5)	52 (60.5)	1.54 (0.93–2.53)	0.092
No	288 (77)	86 (29.9)	202 (70.1)		
Living in over crowded house					
Yes	268 (71.7)	76 (28.4)	192 (71.6)	0.56 (0.35–0.89)	0.014
No	106 (28.3)	44 (41.5)	62 (58.5)		
Exposure to wood biofuel					
Yes	331 (88.5)	117 (35.3)	214 (64.7)	7.29 (2.21–24.1)	0.001
No	43 (11.5)	3 (7)	40 (93)		
School attendee					
Yes	92 (24.6)	48 (52.2)	44 (47.8)	0.31 (0.19–0.51)	< 0.001
No	282 (75.4)	72 (25.5)	210 (74.5)		
Total	374 (100)	120 (32.1)	254 (67.9)		

The proportion of culture confirmed bacterial tonsillitis was higher among children who had history of contact with coughing patients (61%) than the counters (24%). Daycare center attendee children had a higher (39.5%) percentage of bacterial tonsillitis than others (29.9%). Moreover, school attending children had a higher (52.2%) percentage of bacterial tonsillitis than the counterparts (25.5%). The proportion of bacterial tonsillitis was higher among children who had exposure to biofuel (35.3%) than others (7%) (Table 3).

Distribution of bacteria isolates

A total of 143 (32%) bacterial pathogens were isolated from 120 culture positive samples. The most frequent isolate was *S. pyogenes* 78 (55%) followed by *S. aureus* 42 (29%) and *S. pneumoniae* 10 (7%) (Fig. 1). Among the 120 children with confirmed bacterial tonsillitis, 23 (19.2%) had mixed infections. *S. pyogenes* and *S. aureus*, *S. pneumoniae* and *S. aureus* and *S. aureus* and *M. catarrhalis* were the most common mixed isolates with a proportion of 10 (8.3%), 4 (3.3%) and 4 (3.3%), respectively (Table 4).

Table 4
Distribution of mixed isolates and MRSA from the total culture confirmed bacterial tonsillitis (n = 120) among children within five years of age at Hargeisa Group of Hospital

Type of mixed isolates	Frequency (%)
<i>S. pyogenes</i> + <i>S. aureus</i>	10 (8.3)
<i>S. pyogenes</i> + <i>P. aeruginosa</i>	3 (2.5)
<i>S. pneumoniae</i> + <i>S. aureus</i>	4 (3.3)
<i>S. pneumoniae</i> + <i>K. pneumoniae</i>	2 (1.7)
<i>S. aureus</i> + <i>M. catarrhalis</i>	4 (3.3)
Total	23 (19.2)
MRSA	19 (15.8%)
Key: MRSA: Methicillin resistant <i>S. aureus</i>	

Antibiotic resistance profiles of bacterial isolates

Overall, 131 (91.6%) bacterial isolates were resistant to ampicillin. Relatively, higher resistance percentages were found against gentamicin (41.3%), ofloxacin (34.3%) and clarithromycin (32.2%). *S. pyogenes*, *S. aureus*, *S. pneumoniae* and *M. catarrhalis* isolates revealed an overall resistance of 33.5%, 37.2%, 28.8% and 50%, respectively. *S. pyogenes* revealed resistant to ampicillin (94.9%), ofloxacin (43.6%), and gentamicin (42.3%). *S. aureus* isolates showed resistance to ampicillin (83.8%) clarithromycin (38.1%), and ciprofloxacin (35.7%). The percentage of *S. pneumoniae* isolates resistance to ampicillin, gentamicin, clarithromycin and erythromycin was 100%, 60%, 30%, and 30%, respectively. *Klebsiella pneumoniae* isolates were resistant to ampicillin (83.3%) and erythromycin (66.7%). All *P. aeruginosa* isolates were resistant to ciprofloxacin and ampicillin (Table 5).

Table 5

Antibiotic resistance profile of bacterial isolates from children under the age of five with tonsillitis at Hargeisa Group of Hospital

Antibiotics tested	<i>S. pyogenes</i> (n = 78)		<i>S. aureus</i> (n = 42)		<i>S. pneumoniae</i> (n = 10)		<i>M. catarrhalis</i> (n = 4)		<i>P. aeruginosa</i> (n = 3)		<i>K. pneumoniae</i> (n = 6)		Total (n = 143)	
	#T	R%	#T	R%	#T	R%	#T	R%	#T	R%	#T	R%	#T	R%
Amoxicillin-clavulanic acid	78	6 (7.7)	42	10 (23.8)	10	0	4	2 (50)	3	1 (33.3)	6	2 (33.3)	143	21 (14.7)
Ciprofloxacin	78	8 (10.3)	42	15 (35.7)	10	0	4	2 (50)	3	3 (100)	6	2 (33.3)	143	30 (21)
Clarithromycin	78	22 (28.2)	42	16 (38.1)	10	3 (30)	4	2 (50)	3	1 (33.3)	6	2 (33.3)	143	46 (32.2)
Gentamicin	78	33 (42.3)	42	13 (31)	10	6 (60)	4	3 (75)	3	2 (66.7)	6	2 (33.3)	143	59 (41.3)
Vancomycin	78	10 (12.8)	42	14 (33.3)	10	0	4	0	3	1 (33.3)	6	2 (33.3)	143	27 (18.9)
Ofloxacin	78	34 (43.6)	42	11 (26.2)	10	1 (10)	4	0	3	2 (66.7)	6	1 (16.7)	143	49 (34.3)
Erythromycin	78	22 (28.2)	42	11 (26.2)	10	3 (30)	4	3 (75)	3	2 (66.7)	6	4 (66.7)	143	45 (31.5)
Ampicillin	78	74 (94.9)	42	35 (83.3)	10	10 (100)	4	4 (100)	3	3 (100)	6	5 (83.3)	143	131 (91.6)
Total	624	209 (33.5)	336	125 (37.2)	80	23 (28.8)	32	16 (50)	24	15 (62.5)	48	20 (41.7)	1144	408 (35.7)

#T: number of isolates tested, R%: percent of isolates resistant to antimicrobial agents

Multiple Drug Resistant (MDR) profiles of bacterial isolates

Overall, 72 (50.4%) of the bacterial species were MDR and 52.6% of *S. pyogenes* were MDR. The MDR profile of *S. aureus*, *S. pneumoniae* and *Klebsiella pneumoniae* isolates were 18 (42.9%), 6 (60%) and 3 (50%), respectively (Table 6).

Table 6

Multidrug resistance profiles of bacterial isolates from children within five years of age with tonsillitis at Hargeisa Group of Hospital.

Bacterial species	R1 N (%)	R2 N (%)	R3 N (%)	R4 N (%)	R5 N (%)	R6 N (%)	Over all MDR N (%)
<i>S. pyogenes</i> (78)	16 (20.5)	21 (26.9)	24 (30.3)	14 (17.9)	3 (3.8)	0	41 (52.6)
<i>S. aureus</i> (42)	8 (19)	16 (38)	2 (4.8)	7 (16.7)	5 (11.9)	4 (9.5)	18 (42.9)
<i>S. pneumoniae</i> (10)	4 (40)		5 (50)	1 (10)	0	0	6 (60)
<i>K. pneumoniae</i> (6)	1 (16.7)	2 (33.3)	1 (16.7)	2 (33.3)	0	0	3 (50)
<i>M. catarrhalis</i> (4)	1 (25)	2 (50)	1 (25)	0	0	0	1 (25)
<i>P. aeruginosa</i> (3)	0	0	2 (66.7)	1 (33.3)	0	0	3 (100)
Total (143)	30 (20.9)	41 (28.7)	35 (24.5)	25 (17.5)	8 (5.6)	4 (2.8)	72 (50.4)

R0: Susceptible to all classes of antibiotics, R1, R2, R3, R4, R5, R6: resistant to 1, 2, 3, 4, 5, and 6 antibiotic classes, MDR: Resistance of an isolate to three or more antibiotics taken from different classes

Multivariable analysis

Based on multivariable analysis, bacterial tonsillitis was significantly associated with difficulty of swallowing (AOR = 6.99, CI = 3.56–13.13), weight loss (AOR = 0.33, CI = 0.186–0.597), attending school (AOR = 2.98, CI = 1.64–5.42), history of tonsillitis (AOR = 0.12, CI = 0.06–0.21) and exposure to biofuel (AOR = 0.19, CI = 0.04–0.84). Children who had difficulty of swallowing were 7 times more likely to become culture positive for bacterial tonsillitis, compared to children who did not have difficulty of swallowing. Likewise, school attending children were 3 times more likely to have confirmed bacterial tonsillitis compared to non-attendees. Children with history of tonsillitis were more likely to had bacterial tonsillitis than those without history of tonsillitis. Similarly, children who had weight loss and exposure to biofuel were more likely to become culture confirmed tonsillitis compared to those who did not have weight loss and exposure to biofuel (Table 7).

Table 7
Multivariable analysis of factors associated with bacterial tonsillitis among children under the age of five years at HGH

Variables	COR (95%CI)	P-value	AOR (95%CI)	P-value
Gender				
Male	0.64 (0.41–1.00)	0.05	0.68 (0.38–1.19)	0.18
Female				
History of tonsillitis				
Yes	0.11 (0.68 – 0.19)	< 0.001	01.2 (0.06–0.21)	< 0.001
No				
Type of tonsillitis				
Acute				
Chronic	8.37 (4.82–14.53)	< 0.001		
Recurrent	0.87 (0.45–1.67)	0.67	0.45 (0.01–17.2)	0.67
Parental occupation				
Unemployed	1.98 (1.19–3.29)	0.008	1.68 (0.89–3.18)	0.11
Employed				
Contact with cough				
Yes	1.89 (1.18–3.0)	0.008	0.71 (0.37–1.33)	0.29
No				
Tonsillar stricture				
Yes	3.52 (1.93–6.42)	< 0.001	2.1 (0.05–80.7)	0.69
No				
Over crowded				
Yes	0.56 (0.35–0.89)	0.02	0.93 (0.45–1.91)	0.93
No				
Difficulty of swallowing				
Yes	0.32 (0.198–0.53)	< 0.001	6.99 (3.56–13.73)	< 0.001
No				
Weight loss				
Yes	2.96 (1.89–4.64)	< 0.001	0.33 (0.186–0.597)	< 0.001
No				
Attending school				
Yes	0.31 (0.19–0.51)	< 0.001	2.98 (1.64–5.42)	< 0.001
No				
Attending day care center				
Yes	1.54 (0.93–2.53)	0.09	1 (0.44–2.34)	0.97
No				

Variables	COR (95%CI)	P-value	AOR (95%CI)	P-value
Wood biofuel				
Yes	7.29 (2.21–24.1)	0.001	0.19 (0.04–0.84)	0.029
No				

Discussion

Tonsillitis has considerably a negative impact on the patients' quality of life and has a significant burden on public health. Untreated childhood tonsillitis can lead to peritonsillar abscess, tonsillar stones, and rheumatic fever. Therefore, identification and antimicrobial susceptibility of bacterial causes of tonsillitis is essential to curtail for the treatment of tonsillitis. However, patients with tonsillitis managed empirically in health care's settings of Somaliland. Therefore, this study presents the first report of the prevalence of culture confirmed bacterial tonsillitis and the antimicrobial resistance profiles of isolates in HGH.

In this study, 32.1% of children within five years of age had culture confirmed bacterial causes of tonsillitis. Due to the lack of previous data in Somaliland, comparison of countrywide results was not possible. However, the prevailing magnitude of tonsillitis is higher than similar studies with a prevalence of 11.3% in Ethiopia [8], 20.6% in Tanzania [1], 21.6% in Norway [9], and 19% in Bangladesh [10]. On the other hand, the existing prevalence of bacterial causes of tonsillitis from this study was lower than studies done in the United Kingdom (79%) [11], Trinidad (62.5%) [12], India (72%) [4], Saudi Arabia (65%) [13], Benin (73.97%) [5] and Ethiopia (51%) [14]. The lower rate of bacterial tonsillitis in the present study compared to other developing countries might be attributed to differences in geography, community living status and hygienic practices, host factor and educational level of the parents.

The prevalence of bacterial causes of tonsillitis in children within five years of age was higher in males than in females which is similar to studies from India [2] and Nigeria [15]. The variations on the percentage of tonsillitis between genders of the children could be due to the fact that males spend more time in outdoor than females. The percentage of tonsillitis was higher among children living in urban than rural areas. This was similar with studies done in India [2], and Ethiopia [14]. This might be due to variation in: encountering infected people, exposure with air pollution from biofuel use, schooling and house crowding.

High rate of bacterial tonsillitis was reported among patients symptomatic for chronic (57.7%) and recurrent tonsillar infections (51.2%). These are indications of antimicrobial resistance and tonsillectomy. Regarding the prevalence of bacterial isolates; *Streptococcus pyogenes* was the most frequent bacterial isolate from children with tonsillitis in this study and its percentage (55%) is similar with studies in Ohio (58%) [16], Italy (69%) [17], and Trinidad (82.2%) [12]. However, it is higher than studies from Egypt (17%) [18], Iran (20%) [19], Iraq (29.7%) [20], India (22.25%) [21], Saudi Arabia (40%) [22], and Ethiopia (12.2%) [23]. This variation might be influenced by climate changes, age and geographical inhabitation of the study participants.

In the present study, *Staphylococcus aureus* was the second most frequent isolate of bacterial causes of tonsillitis with a rate of 29%. This could be due to the persistence of *S. aureus* in the tonsillar tissues, treatment with antimicrobials and antibiotic resistance. Moreover, *S. aureus* has the potential to form biofilm which results recurrent and chronic infection as well as treatment failure. The isolation of *S. aureus* as the main agent of tonsillitis has been reported by several authors in Ethiopia [16], Brazil (40%) [24], Trinidad (68.9%) [12], and Nigeria (32.1%) [15].

It is a fact that isolation of *S. pneumoniae* indicates the existence of recurrent tonsillitis in children. The percentage (7%) of *Streptococcus pneumoniae* isolates from children with tonsillitis in the present study is lower than studies done from Poland (14%) [25], Belgium (21%) [26], Italy (4%) [17], and South Ethiopia (62.5%) [27]. However, it was higher than studies done in the US (3.5%) [28], Nepal (4%) [29] and Nigeria (3.3%) [30]. The percentage (4%) of *Klebsiella pneumoniae* causing tonsillitis in this study is higher than a study done in Brazil (1.4%) [31], but was lower than studies done in Singapore (6.6%) [32], Indonesia (7%) [33]. In this study, the prevalence of *Moraxella catarrhalis* was 3% which is different from studies done in the USA (22%) [34], Brazil (28.5%) [35], Denmark (53%) [36], Tanzania (90.8%) [37], and Ethiopia (12.3%) [14].

In the present study, there is high proportion of mixed infections particularly with *S. pyogenes* and *S. aureus*, *S. pneumoniae* and *S. aureus* and *S. pneumoniae* and *M. catarrhalis*. These co-infections of the tonsils may contribute to the severe inflammatory process

and the failure of penicillin and ampicillin therapy which finally results recurrent infection, tonsillectomy, rheumatic fever and other complications [34, 38].

In this study, the percentage of Methicillin Resistant *S. aureus* (MRSA) isolates among children with tonsillitis was 15.8%. This is higher than studies done in Germany (0.8%) [37], Lahore (5.5%) [39], Japan (0.8%) [40], Brazil (3.3%) [24] and Ethiopia (2.3%) [41], but lower than studies done in the USA (16%) [42], Benin (17.95%) [5] and Uganda (32%) [43]. The variation between the studies might be due to geographical variations, age and child contact to hospitalized patients who could have contracted the MRSA from hospitals.

The resistance of the isolates to ampicillin was 91.6% and 14.7% to the association of amoxicillin and clavulanate. The higher resistance to ampicillin by all of the bacterial isolates might be due to production of beta lactamase enzyme as well as abuse and excessive use of cheap drugs, which can be afforded and administered without a physician's guidance. This is a major concern that limits the use of this common therapeutic option in clinical practice in developing countries. The rate of penicillin resistance is comparable with reports from Nigeria (100%) [5].

The resistance rate of 94.9% of *S. pyogenes* to ampicillin is worrisome. As B-lactam antibiotics are the drug of choice for strep throat. The percentage of *S. pyogenes* resistant to gentamicin (42.3%) and ofloxacin (43.6%) in the present study was comparable to studies done in Iran (32.2%) [44]. The resistance of *Streptococcus pyogenes* to the above drugs might be due to the enzymatic inactivation mediated by aminoglycoside-modifying enzymes (AMEs), and point mutations in the quinolones resistance-determining region (QRDR).

The resistance of *S. aureus* to ciprofloxacin (35.7%) in this study, was lower than studies done in Egypt (90.9%) [45] and Nepal (100%) [46], but was higher than studies done in Brazil (24.6%) [24]. On the other hand, the resistance profile of *S. pneumoniae* to erythromycin (30%) is similar to a study done in Malaysia, with a rate of (30%) but different from studies done in China (56%, 20%) [47], Lithuania (78.8%) [48] and Ethiopia (12.4%) [49].

One of the major worries when determining resistance profiles of isolates is the availability of MDR strains. In this study, half of the bacterial isolates were MDR. This is a serious problem for children within five years of age in Somaliland. Children involved in the study area were outpatients and they might have constant contact with other children and their family. Moreover, in the study area there is no routine culture and antimicrobial susceptibility testing and management of children with tonsillitis is empirical. These may result in repeated infections of the tonsils, pyogenic meningitis, rheumatic fever, lower respiratory tract infections and difficulty to select the effective antibiotics. Furthermore, existence of MDR isolates demonstrates persistence of the bacteria and possibility of antimicrobial resistance dissemination and recurrence of infection [34].

The percentage of MDR *S. pneumoniae* (60%) in this study was higher than studies from Poland (52.9%) [50], Lithuania (12.5%) [48] and Vietnam (35%) [51]. In this study, all isolates of *Pseudomonas aeruginosa* were MDR (100%) which is concurrent to a study in Brazil (100%) [31]. These high proportions of MDR among the isolates might be due to production of beta-lactamase enzyme by *Pseudomonas aeruginosa* and production of Penicillin binding proteins in *Streptococcus pneumoniae*.

In the present study, difficulty of swallowing is one of the predictors of bacterial tonsillitis in children within five years of age. Similar findings were reported in India [2] and Lithuania [48]. History of tonsillitis was also a predictor variable in this study which was similar to studies done in Ethiopia [9], and Yemen [52]. These might be due to cohabitations of the tonsils by multiple bacterial isolates as depicted in Table 4 and failure of penicillin and ampicillin therapy.

Weight loss was also another predictor for bacterial tonsillitis in this study in which similar studies were reported in Iran [53], and Germany [54]. Furthermore, attending school was a risk factor for tonsillitis in this study similar to studies done in Uganda [55], and Australia [56]. This might be due to overcrowding during schooling among children where carrier children can easily interact with healthy children.

Limitations Of The Study

This study provided the first report of data on bacterial causes of tonsillitis and antibiotic resistance profiles of the isolates from children within five years of age with tonsillitis at Hargeisa Group of Hospital. However, the study limited to identifying nonbacterial causes of tonsillitis.

Conclusions

High prevalence of tonsillitis with MDR pathogens, MRSA and mixed isolates were found. *S. pyogenes* followed by *S. aureus* and *S. pneumoniae* were the most frequent isolates. Most of the bacterial isolates were resistant to ampicillin. However, amoxicillin-clavulanic acid and ciprofloxacin are the least resisted drugs. Therefore, the result points the significance of culture and antimicrobial susceptibility testing in the diagnosis and treatment of any form of tonsillitis for the selection of the effective antibiotics against several pathogenic agents of tonsillitis and rational use of antimicrobials.

Further investigation to identify nonbacterial causes of tonsillitis, conducting studies covering larger geographical areas to draw the magnitude and topographic variations are needed to control the spread of tonsillitis among children within five years age.

Abbreviations

AMEs

Aminoglycoside-Modifying Enzymes

AST

Antimicrobial Sensitivity Testing

ATCC

American Type Culture Collection

AOR

Adjusted Odd Ratio

BA

Blood Agar

CA

Chocolate Agar

CDC

Centre for Disease Control

CFU

Colony Forming Unit

CLSI

Clinical and Laboratory Standard Institute

CMHS

College of Medicine and Health Science

COR

Crude Odd Ratio

ENT

Ear Nose and Throat

HGH

Hargeisa Group of Hospital

IRB

Institutional Review Board

MAC

MacConkey agar

MDR

Multi-Drug Resistant

MHA

Mueller Hinton Agar

MRSA

Methicillin Resistant *Staphylococcus aureus*

MSA

Manitol Salt Agar

QRDR

Declarations

Ethical approval and consent to participate

This study was approved by the institutional review board (IRB) of College of Medicine and Health Science (CMHS), Bahir Dar University and a permission letter was obtained from the Ministry of Health, Somaliland, and Hargeisa Group of Hospitals (HGH). In addition, written informed consent was obtained from parents/guardians of participating children before collecting data. Information obtained from study participants was kept confidential and used only for the purpose of this study. Bacteriologically positive results were communicated with the hospital for better management.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

Availability of data and materials

The data supporting the conclusion of the study are included in the manuscript

Funding

Bahir Dar University financially supports to conduct this study. The funder had no role in the overall design and conduction of the study.

Authors' contributions

HHD: Concept and designed the study, performed the laboratory work, collected and managed the data, analyzed and interpreted the results and contributed to the scientific content of the manuscript. AM: supervised and facilitated the data collection and management, interpreted results, drafted the manuscript and significantly contributed to the scientific content of the study. MK: interpreted results, drafted, critically reviewed, edited and significantly contributed to the scientific content of the manuscript. WM designed and supervised the study, facilitated the data collection and management, analyzed data, interpreted results, drafted the manuscripts and significantly contributed to the scientific content of the study. All authors read and approved the final manuscript.

Acknowledgements

Our gratitude goes to Bahir Dar University, College of Medicine and Health Sciences, Department of Medical Laboratory Science for the opportunity to undertake this study and the provision of ethical clearance. We also thank the parents/guardians of study participants. The insightful guidance of Mr. Ahmed Hashi (head of Laboratory at Hargeisa Group of Hospital) and the technical support from Mr. Mukhtar Botan and Ms. Ugbad Ibrahim at Hargeisa Group of Hospital are also acknowledged.

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Figures

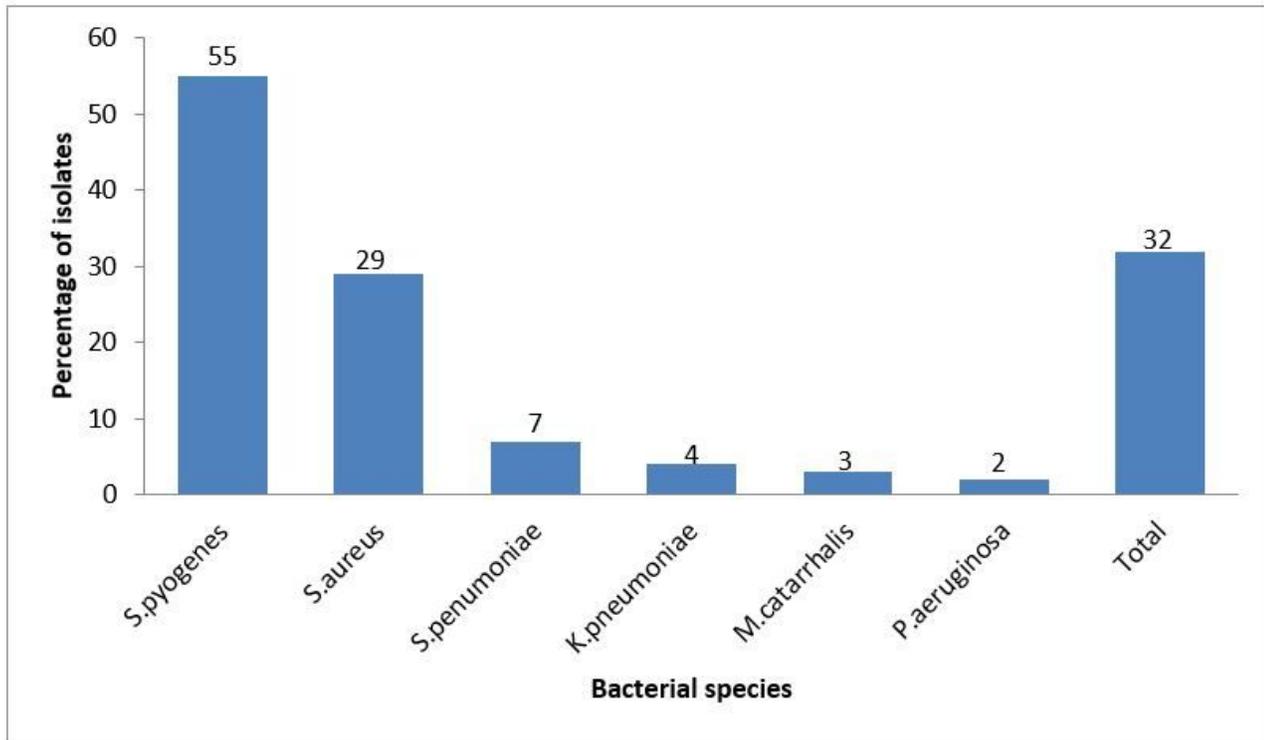


Figure 1

Frequency of bacterial species isolated from Children within five years of age with tonsillitis.