Al-Mustansiriyah Journal of Science
ISSN: 1814-635X (print), ISSN:2521-3520 (online) Volume 33, Issue 5, 2022 DOI: http://doi.org/10.23851/mjs.v33i5.1306

Research Article **Open Access**

Molecular Identification of Prevalent Streptococcus Pyogenes Serogroup Associated with Respiratory Tract Infections in Children

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Article Info

Received
15/12/2022

Accepted
15/01/2023

Published
25/02/2023

ABSTRACT

The current study intends to characterize the respiratory tract infections that have an association with gram positive bacterial pathogens, *emm* typing, the pattern of antibacterial resistance in isolated pathogens, phenotyping of virulence factor and molecular detection of Macrolide resistance gene. Various samples from patients with respiratory tract infections were collected and identification and antimicrobial susceptibility of clinical isolates was performed as per standard laboratory procedure. Macrolides (Eruthromycin, Clindamycin)-resistant isolates were again subjected to MIC method. Macrolide-inducible resistance to clindamycin (D test) was conducted upon Erythromycin and Clindamycin discs. Multiplex PCR was used to detect the resistance genes in all the three types of macrolide resistance strains. Serum Opacity Factor (SOF) was detected for all the isolates of GAS. Every isolate was checked to produce biofilm through micro titre plate method. Bacterial growth got registered in 156 (36.28%) samples. The most common isolate from URI samples was GAS i.e., 64 (14.9%), only to be followed by GGS 38(8.8%), GCS 29 (6.7%) and Staphylococcus aureus 18 (4.2%). Among GAS, only one isolate was recorded from blood, whereas 8.50% from sputum and the rest. All 71 GAS isolates were found to exhibit sensitivity towards Penicillin and Ceftriazone. GAS exhibited 55% Mtype of resistance whereas 40% were resistant to cMLS and 5% to iMLS. GCS showcased an equal number of cMLS and M type too. GGS portrayed 54.54% resistance to cMLS followed by 36.36% to Mtype and 9.09% to iMLS. The current study found iMLS type with least resistance. The current study identified that *Streptococcus pyogenes* is the most common bacteria that cause and recur the infection. The prevalence of resistance tends to change geographically and periodically. For this purpose and to achieve a sound public health outcome, periodical screening of antibiotic-resistance pattern becomes inevitable.

KEYWORDS: *Streptococcus pyogenes*; Respiratory infection; GAS; antibiotic-resistance pattern.

الخلاصة

تشمل الدراسة محاولة توصيف امراض الجهاز التنفسي الناجمة عن الأصابة بالبكتريا المرضية الموجبة لصبغة كرام من النوع (emm type) مع دراسة نمط حساسيتها لمضاداة الحياة المرتبطة بالصفات المظهرية وعوامل الضراوة مع التشخيص الجزيئي للجين المسؤول عن مقاومة الماكروليدات (Macrolide resistance) في البكتريا المعزولة. تم اخذ نماذج مختلفة من المرضى المصابين والمشخصين بالتهاب الجهاز التنفسي, حيث تم جمع العزلات المرضية وتشخيصها مع عمل فحص الحساسية لمضاداة الحياة لها باعتماد المعايير والطرائق المختبرية القياسية لها. كما تم اجراء فحص (التركيز المثبط الأدنى MIC) لمضادات الماكروليدات (Erythromycin and Clindamycin) للعزلات المقاومة لهذه المضادات. حيث تم اجراء فحص مقاومة الماكروليدات المحتث (Macrolide-inducible resistance to clindamycin) بواسطة فحص الأنتشار البسيط (D-test) باستخدام اقراص المضادين (Erythromycin and Clindamycin discs). تم استخدام طريقة تفاعل البلمرة المتسلسل متعدد الإرسال (Multiplex PCR) للكشف عن جينات المقاومة للسلالات الثلاثة, كما اجري اختبار عامل عتامة المصل (SOF) لكل العزلات المشخصة ضمن المجموعة (A) (GAS) كما اجري الكشف عن تكوين الغشاء الحيوي (Biofilm) لكل عزلة بكتيرية بطريقة (micro titre plate). تم تشخيص النمو البكتيري في 156 عينة (36.28%) حيث اظهرت ان اغلب العزلات من اعلى الجهاز التنفسي من نوع (GAS) وهي 64 عينة (14.9%) تليها مجموعة (GGS) 38 (8.8 %), مجموعة (GCS) 29 (6.7 %) والمكورات العنقودية الذهبية 18 (4.2 %), كما تبين ان هناك عزلة واحدة فقط تم عزلها من الدم هي ضمن المجموعة (GAS). كل عزلات المجموعة (GAS) (71) عزلة قد اظهرت حساسية تجاه المضادات (Penicillin , Ceftriazone) كما وجد ان 55% منها من نوع المقاومة (Mtype) و 40 % منها من نوع (cMLS) و 5 % منها من نوع (iMLS). كذلك اظهرت المجموعة (GCS) نسب متساوية بين نوعي المقاومة (cMLS, M type), مناصفة. اما المجموعة (GGS) فقد تم تسجيل 54.45 % نوع (cMLS), 36.36 نوع (Mtype) و9.09%

(iMLS). وجد من خلال هذه الدراسة ان بكتريا *Streptococcus pyogens* هي المسبب المرضي الأكثر شيوعا بين الأصابات كما تم تحديد نوع المقاومة (iMLS type) هي من اقل الانواع مقارنة بالأخريات حيث ان وجود المقاومة الجرثومية للمضادات الحياتية متغيرة مناطقيا وزمنيا, ولهذا السبب ولتحقيق واقع صحي افضل وجد ان عملية اجراء مسح بايولوجي لمقاومة مضادات الحياة بصورة مستمرة يعطي نتائج شفاء واصابات اقل خطورة مستقبلا.

INTRODUCTION

In developing countries, Respiratory Tract Infection (RTI) is one among the major health threats that specifically increases the mortality rate among infants and children [1][2]. RTI remains the primary cause behind 50% of healthcare facility visits and 40% of admission in hospitals [3]. RTI is caused by microbes among which the most cases are reported with viral aetiology and bacterial strain, *Streptococcus pyogenes* (*S.pyogenes*) [4]. Both virus and bacteria are observed in RTI owing to which it becomes inevitable to distinguish the causal microbe since 'bacterial causes' exhibit good response towards antimicrobial therapy [4]. *S. pyogenes* induces local and systematic invasive infections in association with immunologically mediated Post Streptococcal sequelae. So, the focus of current study is upon infection caused by *S. pyogenes* and other such beta haemolytic Streptococci. M protein is major virulence factor of the organism which is highly conserved among Group A isolates There is almost no or little M protein present in streptococci, isolated from chronic pharyngeal carriers and can be said as relatively avirulent [5][6]. An antiphagocytic effect is mediated by M protein in non-immune host by inhibiting the alternate complement pathway, from getting activated. The acquired immunity, in case of streptococcal infection, is based on opsonic antibody development and is targeted towards the antiphagocytic epitopes of M protein. Community based outbreaks of streptococcal diseases tend to be associated with certain M types; therefore, M serotyping has been very valuable for epidemiological studies. So far, the researchers have recognized more than 80 M protein types [7]. So, it is important to have a typing scheme that determines and characterizes the genetic diversity among *S. pyogenes'* isolates. Emm gene sequence encodes the M protein whereas the former is the basis for emm typing and the latter is the basis for serological typing scheme [8].

At most of the times, antibiotic therapy not only fails to get rid of bacterial infection but also aggravates chronic throat carriage and recurrent infection [9][10][11]. Streptococcal infection often fails to antibiotic therapy, leading to chronic recurrent infection. Although rate of antibiotic resistance is very less in *S. pyogenes*, but recent studies suggests that some subtypes of *S. pyogenes* produce biofilm and may have a role in chronic carriage of *Streptococcus*. However, the primary reason may not be antibiotic resistance since *S. pyogenes* exhibit very less resistance towards antibiotics than other bacterial strains. Research studies conducted so far argues that biofilm is produced by few sub-strains of *S. pyogenes* which plays an important role in chronic carriage of *Streptococcus* [12]. The antibiotic rresistance of *S. pyogenes* or in general *streptococcus* species, is evenly susceptible to Penicillin. On the other hand, if the patient is allergic to penicillin, another drug named Macrolides is preferred [13]. The current study intends to characterize the respiratory tract infections that have an association with gram positive bacterial pathogens, *emm* typing, the pattern of antibacterial resistance in isolated pathogens, phenotyping of virulence factor and molecular detection of Macrolide resistance gene.

MATERIALS AND METHODS

Sample collection

Being a prospective and descriptive study, the current research work was conducted between January 2018 and December 2020 for a period of 2 years at Baghdad. This is a tertiary care at Baghdad teaching hospital, located in Baghdad Iraq. This hospital primarily provides healthcare and support services to sub urban locality nearby the hospital. So, the study included a mixed population containing suburban and rural people.

Sample size was estimated to be 600. Sample size was calculated from the pilot study done for three months in the same center by researcher, which showed a prevalence of 5% of bacterial etiology in RTI. The study population was inclusive of all the children aged between 0 and 14 years, who visited the paediatric department at teaching hospital during the study period with respiratory tract infection (RTI) complaints. Exclusion criteria of the study were those patients with chronic respiratory ailments and those patients whose parents did not provide consent for participation in the study. After obtaining necessary approval from

institutional ethical clearance board, informed consent was obtained from the parent of the children. A detailed case study was developed for every patient submitted in this healthcare centre that covers information on demographics, anthropometry, vaccination and clinical history determined by provisional diagnosis by medical staff. With regards to specimen collection for upper RTI, oral cavity of the patients with tonsillitis and pharyngitis was checked using a tongue depressor so as to find the presence of inflammation, any other membranes, exudate or pus. Sterile cotton swab was used to collect the throat swab by rubbing tonsillar and peritonsillar areas. To check the lower RTI in LRI, the researcher collected sputum, tracheal aspirate, pleural fluid and blood samples. Blood was aseptically collected with much precaution and immediately inoculated under aseptic conditions. Then it was transferred to Bacte Alert System, an automated blood culture bottle.

Identification and antimicrobial susceptibility of clinical isolates

All samples were generally cultured on (Blood agar) media for the detection of β-hemolysis, which is followed by subsequent identification steps, and enhances the growth of streptococci by the addition of an external source of catalase. Optimal incubation conditions for the vast majority of streptococcal strains include a temperature range of 35°C to 37°C in the presence of 5% CO2 or under anaerobic conditions. Growth of typical colonies can be observed after 24 hours of incubation at 35-37°C. [25]. After the detection of β-hemolytic colonies displaying a typical *S. pyogene*s morphology, catalase testing confirms that the isolates represent streptococci. All isolates were confirmed by Vitek system instrument at health care center laboratory.

Macrolides (Eruthromycin, Clindamycin)-resistant isolates were again subjected to MIC method. Isolates which are resistant to macrolides (Eruthromycin, Clindamycin) were further subjected by MIC method according to Clinical and Laboratory Standards Institute (CLSI)[14].

Epsilometer Test (E-Test) for Minimum Inhibitory Concentration (MIC)

The test organism turbidity was adjusted to 0.5 McFarland's and with use of a swab, the entire agar surface was evenly swabbed in three directions. E-test strips were placed on dry petridishes and they were incubated for 24hours. The MIC was read where the ellipse intersects the scale at complete inhibition of growth including hazes and isolated colonies.

Double-disk diffusion test (D test) screening

D test was performed with Erythromycin and Clindamycin disc to detect the three phenotypes of MLS [15]. To identify the three phenotypes of MLS, D test was conducted upon Erythromycin and Clindamycin discs [16]. Isolates cultured on Muller Hinton agar plate contain 5% sheep blood to reveal beta hemolytic Streptococci. A Clindamycin (2µg) disk and an Erythromycin (15µg) disk was placed 12mm apart for detecting D- zone testing. Inoculated agar plate was incubated at 35 C in 5% CO2 overnight. iMLS$_B$ were identified where clindamycin zone was blunted towards the Erythromycin as erythromycin induces Clindamycin resistance. cMLS$_B$, was identified with no zone around either Erythromycin or Clindamycin as erm gene was fully expressed and M type showed no change in the Clindamycin zone induced by Erythromycin.

Multiplex PCR to detect the resistant genes

All the three types of macrolide resistance strains were targeted in study and used to screen the genes in erythromycin resistant isolates that encode macrolide resistance. Erythromycin resistance may occur due to an efflux mechanism, M phenotype – *mef*. Erythromycin resistance is acquired through the modification of a target site, mediated by methylase, which in turn changes the 50S ribosomal subunit - MLSB resistance phenotype – *erm* genes. Based on the primers published earlier, *mef*, *ermB*, and *ermTR* genes were screened [15] as illustrated in Table 1.

Table 1. show primers used in this study.

gene	direction	Size (bp)	Primer
*mef*A	Forward	348 bp	5'-AGTATCATTAATCACTAGTGC-3'
	Reverse		5'-TTCTTCTGGTACTAAAAGTGG-3'
*erm*B	Forward	616 bp	5'-CGAGTGAAAAAGTACTCAACC-3'
	Reverse		5'-GGCGTGTTTCATTGCTTGATG-3'
*erm*TR	Forward	206 bp	5'-GCATGACATAAACCTTCA-3'
	Reverse		5'-AGGTTATAATGAAACAGA-3'

***Emm* typing**

Emm typing was conducted for few specific beta hemolytic streptococci isolates. PCR was conducted to detect the *emm* gene. Blasting of the sequenced result was performed based on CDC

protocol for emm typing of *Streptococcus pyogenes*. A first-of-its-kind emm type was found through BLAST search (http://www.cdc.gov/ncidod/biotech/strep/strepblast.htm) to possess 95% sequence identity with other known types of *emm*. Sequencing was performed using Primer: ATTCGCTTAGAAAATTAAAAACAGG.

Serum Opacity Factor Detection

Detection of SOF was done was for all the GAS isolates [17]. All the isolates were sub-cultured in Brain heart infusion broth at 37°C for overnight. Ten µl of culture supernatant was taken in a micro titre plate for testing with 100 µl of horse serum (Hi media) and incubate overnight. Known positive and negative strains of *S. pyogenes* were taken as controls. The plates were read at 490 nm.

Detection of biofilm production

Biofilm formation was checked in microtitre plate as described previously in [18] with some modifications. Each isolate was inoculated into in 5 ml lourie broth and incubated overnight. 300 µl of LB broth was added 20µl of test sample into well, incubated 37 0C overnight. Wells were washed with PBS buffer followed by addition of 250µl of methanol to fix the biofilm. Safrannin was added to coloure the biofilm and then 33 % acetic acid was added to emulsify the biofilm. Well, were rocked for 10 min and read with ELISA reader at 490 nm [18]. Every isolate was checked to produce biofilm through micro titre plate method.

RESULTS AND DISCUSSION

The study included a total of 600 children aged between 0-14 years. The results recorded a prevalence of 37% bacterial aetiology in RTI. Out of the sample population, upper respiratory tract infection was found in 72.9% (253) patients whereas Lower respiratory tract infection was recorded in 27.1% (94) samples. From LRI patients, the researcher collected a total of 54 blood samples and 12 pleural aspirates in addition to sputum. The children, selected for the study, were mostly in the age group of 11-14 years (37.84%) followed by 6-10 years (26.1%), 3-5 years (23.1%) and < 2 years (12.9%). No specific difference was found in terms of male or female children in the age groups considered, as shown in Figure 1 and 2.

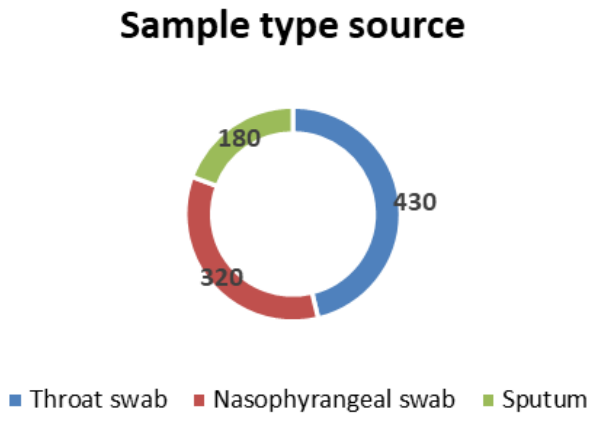

Figure 1. Details of sample type source.

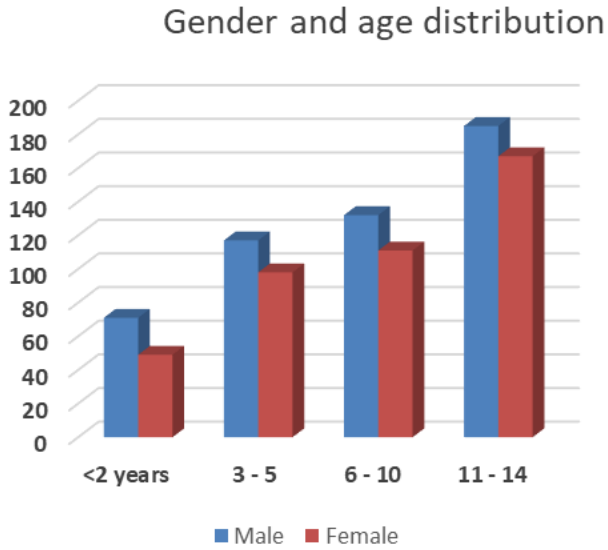

Figure 2. Distribution of age and gender of patients.

The bacterial isolates, collected from a total of 750 URI samples, were assessed. Out of the samples, bacterial growth got registered in 253 (33.73%) samples. The most common isolate from URI samples was GAS i.e., 97 (38.33%), only to be followed by GGS 58 (22.9%), GCS 41 (16.2%) and *Staphylococcus aureus* 31 (12.53%). Rare isolates such as *Hemophilus influenzae* (2), and *Streptococcus pneumoniae* (11) were also found, figure 3. Out of the total 54 blood samples collected from pneumonia cases, bacterial culture was positively found in 21 (38.88%) only, figure 4. This was inclusive of three isolates of *S. pneumoniae* along with 11 GAS as shown in Figure 3.

A total of 133 GAS, 50 GCS and 79 GGS isolates was found among Beta haemolytic Streptococci isolates. Among GAS, only 11 isolates was recorded from blood, whereas the rest were recovered from throat swab. Further, no isolate was recovered from blood among 39 GGS whereas rest were isolated from throat swab. In terms of GCS, all the 29 samples were isolated only from throat swab as shown in Figure 4

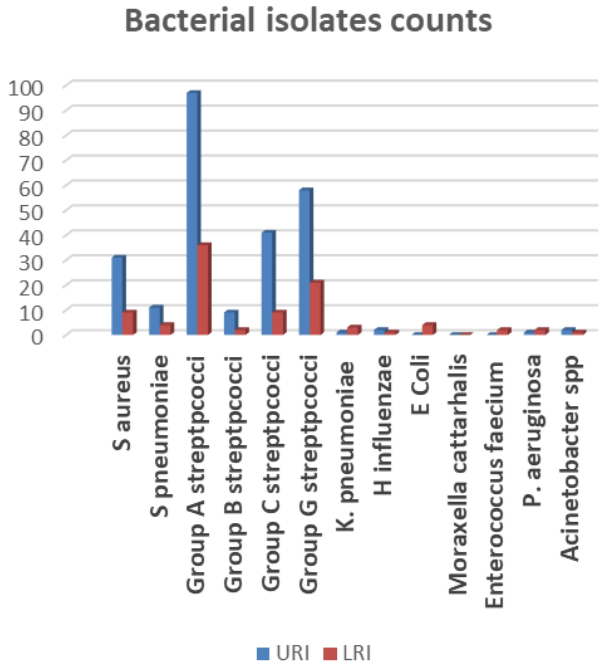

Figure 3. Identification of bacterial isolates.

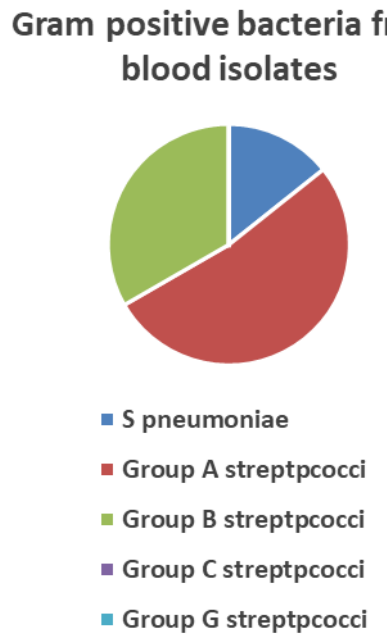

Figure 4. Bacterial identification from blood sample.

In terms of sensitivity, all 71 GAS isolates were found to exhibit sensitivity towards Penicillin and Ceftriaxone. In the perspective of resistance, 41% resisted Erythromycin, 19% showed resistance to Ofloxacin. 15% to Chloramphenicol, 20% to Clindamycin and 87% to Co-trimoxazole. Further, 31% GCS showed resistance to Penicillin, 12% to ceftraizone, 29% to Erythromycin, 23% to Clindamycin and 21% to Co-trimoxazole. Similarly, 40% GGS showed resistance to Penicillin, 13% to Ceftraizone, 37% to Erythromycin, 21% to Clindamycin and 15% to Co-trimoxazole. Double disc diffusion test was conducted for all the isolates with Erythromycin resistance using Erythromycin and Clindamycin discs, (Figure 5). As per the outcomes, GAS exhibited 14% Mtype of resistance whereas 51% were resistant to cMLS and 35% to iMLS. GCS showcased an equal number of cMLS and M type too. GGS portrayed 58% resistance to cMLS followed by 29% to Mtype and 13% to iMLS. The current study found iMLS type with least resistance, as illustrated in Table 2.

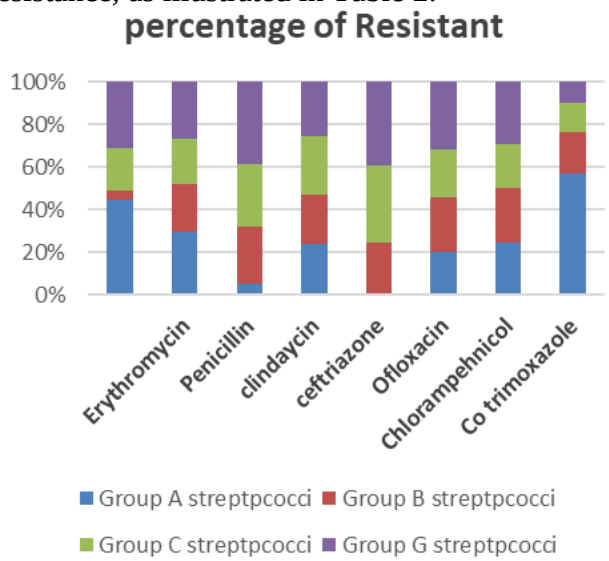

Figure 5. Resistant pattern for streptococci.

Table 2. Minimum inhibitory concentration for erythromycin and clindamycin.

MIC µg/ml	Erythromycin				Clindamycin			
	GAS	GBS	GCC	GGS	GAS	GBS	GCC	GGS
MIC 50	8	16	32	64	8	8	16	32
MIC 90	64	64	126	126	32	64	126	64

Detection of gene encoding macrolide resistance in GAS by multiplex PCR

Multiplex PCR was used to detect the macrolide resistance genes in 11 specific GAS strains. Out of these strains, 5 were M type, 5 were cMLS type and one strain was of iMLS type resistance. A total of three resistance genes was screened for Erythromycin resistance owing to an efflux mechanism. In this, the M phenotype is conferred by a *mefA* gene sized 348bp. Erythromycin resistance is acquired through the modification of a target site, mediated by methylase, which in turn changes the 50S ribosomal subunit - MLSB resistance phenotype – erm genes i.e., *ermA* of 206 bp and *ermB* of 616bp. Out of the five M type resistance strains, only one showed positive for erm B whereas others were positive for *mef A* gene. All

the five c MLS type resistant strains showed positive for *erm B* gene. iMLS type resistant strains were negative, when dealt with phenotypic method D test, for three genes that render macrolide resistance such as *erm A*, *erm B* and *mef A* gene.

Table 3. MLSB resistance phenotype

Resistant phenotype %	Tested	cMLS	iMLS	Mtype
Group A streptococci	41	51	35	14
Group B streptococci	31	65	11	24
Group C streptococci	29	45	9	46
Group G streptococci	37	58	13	29

Emm typing results

In line with CDC protocol framed for emm typing, the procedure was performed. Emm amplicons were of the size range, 800-1500 bp. Gel electrophoresis was conducted to analyze the PCR products in which a 100 bp ladder is utilized. 59 beta hemolytic Streptococci such as 44 GAS, 9GGS and 6 GCS, was subjected to *emm* typing. Among the 44 GAS type, a total of 13 different '*emm* 85' types were identified: *emm* 12.40, *emm* 22, *emm* 25.1, *emm* 42, emm 65.1, *emm* 78.3, *emm* 106, *emm* 113, *emm* 118, *emm* 124, *emm* 152, *emm* 183.2 and *emm* 238.2. The four Group G streptococcus were two each from stG6792.3 and stG6792 while Group C *Streptococcus* was identified as stC5345.1.

Virulence factors analysis

The formation of biofilm was validated for all GAS isolates through micro titre plating method. The researchers verified the formation of biofilm using trypticase soya broth as well as brain heart infusion broth along with 1% glucose while the results were similar in both cases. 55 % isolates produced biofilm. In line with the above, all isolates were again screened for the presence of SOF using horse serum, through micro titre plate method. 73.52% isolates showed the production of SOF i.e., positive outcomes. Fischer's 2X2 contingency table was used to determine the association among biofilm production, SOF and resistance to macrolide. An extremely statistically significant association was found between SOF and biofilm production with P value less than 0.0001. No statistically significant association was found between macrolide resistance and biofilm production since p value was 0.0513.

DISCUSSIONS

Acute rheumatic fever can be prevented through early and precise diagnosis of Streptococcal pharyngitis and proper antimicrobial therapy. Further, these actions also inhibit the supplementary complications (eg, peritonsillar abscess, cervical lymphadenitis, mastoiditis, and, possibly, other invasive infections), enhance the signs and clinical symptoms, reduce the contagiousness drastically, mitigate the transmission of GAS to close acquaintances, family members and friends of the patient, reduce the adverse effects of improper antimicrobial therapy and help the patient to resume their day-to-day activities [4]. The focus of the current study is to study the prevalence of bacterial agents that cause RTI in children and the patterns of antibiotic resistance among patients reporting at the study hospital. The current study reported 231 cases out of 750 (30.8%) as positive for bacterial pathogens that cause acute respiratory tract infection (ARI). In a Brazilian cross-sectional study conducted upon children less than five-years old, ARI was reported in 25.6%, out of which upper respiratory infection was found among 76.4% patients whereas it was 23.6% in case of lower respiratory infections [19]. Few countries such as India, Bangladesh, Indonesia and Nepal contribute 40% of the deaths due to respiratory infection across the globe. ARI is responsible for 30-50% of visits to health facilities whereas hospital administration cause 20-40% infection [1]. Viral infections have been established as the primary cause behind mild-to-moderate pneumonia at initial stages of life whereas severe pneumonia is a direct result of bacterial infections in later stages [20]. In current study, the most common isolate found among URI positives was Streptococcus pyogenes (41%), while the outcome is in alignment with that of the study result attained in Pakistan (44.4%) [21]. GAS contributed 30.7% (71), GCS was 12.5% (29) and GGS was 16.9% (39) out of the total positives. A study conducted in India during 2010 showed only 22.2% prevalence of other beta hemolytic Streptococci [22]. But the current study results infer the presence of high beta haemolytic Streptococci i.e., 43% (GGS 24.4% and GCS 18.6%).

Emm typing was conducted in order to identify the type of GAS clone present in study location. Out of the selected 30 GAS isolates, 12 random strains were macrolide sensitive and macrolide resistant. While 6 strains exhibited unique characteristics

such as 96 cross reactivity to Group C and G types and bacitracin resistance. In line with this, 30 beta hemolytic Streptococci was selected to conduct emm typing. Out of this numbers, 25 were found to be GAS while one isolate remained unamplified and four more were other hemolytic Streptococci. Out of these 25 GAS typed ones, a total of 15 different emm types was identified as given herewith; emm 12.40, *emm* 22, *emm* 25.1, *emm* 42, *emm* 65.1, *emm* 78.3, *emm* 81.11, *emm* 106, *emm* 113, *emm* 118, *emm* 118.5, *emm* 124, *emm* 152, *emm* 183.2 and *emm* 238.2. Some of the typed strains of GGS were stG6792.3, stG6792.2, stG6792 whereas GCS was found to be stC5345.1. The typed *Streptococcus pyogenes* such as *emm* 118, *emm* 106, StC 5345, stG 6792 and stG 672.1 isolated was in line with the study conducted earlier at Chennai. This study was authored by T. menon which had *emm* 118(1 no), *emm* 106 (1 no), StC 5345 (4 number) StG 6792(1no) and StG 6792.1(3 no) [23]. One more study conducted in South India exhibited emm and emmL types, inclusive of *emm* 12.0 (28.6%), stG643.0 (28.6%), *stC*46.0 (17.0%), *emm* 30.11 (8.5%), *emm*3.0 (2.9%), *emm*48.0 (5.7%), st3343.0 (2.9%), *emm*107.0 (2.9%) and stS104.2 (2.9%). Among these types, *emm* 12.0 was only similar to the current study [23].

In terms of sensitivity, the isolated GAS was sensitive to Penicillin, amoxicillin, Cephalothin and cefuroxime. However, it showed only the following resistance values i.e., 17% to Chloramphenicol, 11.26% to Clindamycin, 12.7% to Ofloxacin and 28.1% resistance to Macrolides. D test was performed to identify the macrolide resisting phenotypes in GAS. The results showed that M type resistance is the most common type with 55% resistance followed by cMLS and iMLS with 25% and 10% resistance respectively. In line with these results, GGS also exhibited 56% M type resistance, 36% cMLS resistance and 8% iMLS resistance. However, GCS exhibited only 8% M type and 10% cMLS without any trace of iMLS. Phenotypical identification of 20 macrolide-resistant GAS strains were categorized as 11 M type strains, 8 cMLS strains and 1 iMLS strain. Macrolide resistance gene was detected in 11 chosen GAS strains out of which Mtype and cMLS types had each 5 strains and iMLS had only one strain in the detection of *ermB* gene, erm TR and *mef A* gene. The whole set of M type resistance strains showed positive for mef A whereas only one showed positive for erm B. In case of cMLS, all exhibited positive for *ermB*. Only one iMLS was proved to be positive phenotypically, though it could not be established as such, by molecular method, since it attained negative outcomes for all three genes. The current study documented two unique genotypes i.e., erythromycin resistance *erm*(B) and mef A. This outcome suggests a possible polyclonal origin of resistance in the geographical area under study and is in alignment with literature reported from India [23] [24].

Microbiologists are surprised towards the failure of antibiotic treatment in getting rid of susceptible microbes in recent times. This novel phenomenon led them to hypothesize the attachment of bacteria in the form of communities, otherwise identified as 'biofilms' [25]. When a bacterial biofilm forms, it causes a variety of health concerns and industrial issues [26]. The biofilm formation can be prevented using the traditional strategy *S. pyogenes* was investigated earlier, and it was found that the microbe can produce biofilm to camouflage the antibiotic treatment as well as host defenses, resulting in repetitive infections [3]. This biofilm, created by *S. pyogenes* can overcome the high antibiotic concentrations (in 10-folds) among pharyngitis patients, bypassing the Minimum Inhibitory Concentration (MIC) for planktonic *S. pyogenes* [3]. SOF (Serum Opacity Factor) of *S. pyogenes* acts as a serotyping tool, virulence factor as well as a pathogenesis factor. Due to its virulence nature, the antisera against SOF has the potential to opsonize SOF-positive streptococci in human blood and safeguard the mice against streptococcal infections [17]. All GAS strains, which were screened for the presence of SOF i.e., 38/71 (53.52%) showed positive. The study conducted earlier in the title 'High Diversity of Group A Streptococcal *emm* Types in an Indian Community', 33 out of 59 isolates (55.9%) showed positive for SOF alike the result achieved in current study i.e., 53.2%. The study conducted earlier upon SOF among GAS isolates from skin showed high production of SOF (83.3%), which was contradictory to current study outcomes i.e., 53.52% variations. This might be attributed to M type variation i.e., two categories of M types such as opacity factor positive and opacity factor

negative. So, it is utilized in the subtyping of GAS isolates.

CONCLUSIONS

The current study identified that *Streptococcus pyogenes* is the most common bacteria that cause and recur the respiratory tract infection. Most of *Streptococcus spp*. can complicate the RTI further like Rheumatic fever, acute glomerulonephritis, endocarditis etc. Correspondingly the current study exhibited significant relationship between the virulence factors such as serum opacity factor (SOF) and the formation of biofilm. However, no significant relationship was found between biofilm formation and macrolide resistance among bacterial isolates. When it comes to biofilm formation, macrolide-sensitive isolates gained prominence than the resistant strains. So, it becomes important to diagnose the streptococcal infection in early stages and begin treatment in order to avoid complications. In spite of the fact that the current study found 28.1% macrolide resistance (for instance, Erythromycin and Azithromycin) in GAS, its usage is still preferred. The prevalence of resistance tend to change geographically and periodically. For this purpose and to achieve a sound public health outcome, periodical screening of antibiotic-resistance pattern, and a combination of antisera against M protein and SOF proteins is dramatically more effective in killing streptococci in addition to antiserum alone, indicating that antibodies against SOF enhance the opsonic efficiency of M protein antibodies becomes inevitable. If you claim that 3rd variables might affect your correlations, tell me what they are and how they would affect your correlations.

Speculate about future directions that research could take to further investigate your question. This might relate back to any weaknesses you have mentioned above (or reasons why the results did not turn out as expected). Future directions may also include interesting next steps in the research.

ACKNOWLEDGMENT

The authors thank Mustansiriyah University (https://uomustansiriyah.edu.iq/), Baghdad, Iraq for its support to complete this work.

Disclosure and Conflict of Interest: The authors declare that they have no conflicts of interest.

Author Contributions: All authors contributed equally in writing original draft preparation, all authors have read and agreed to the published version of the manuscript.

Informed Consent: All patients gave their written informed consents before inclusion.

Research Involving Human and Animals Participants: This study involved the 600 from patients who are suffering from respiratory tract infection in Baghdad hospitals, Baghdad, Iraq.

REFERENCES

[1] U.B. Schaad, S. Esposito and C.H. Razi. Diagnosis and management of recurrent respiratory tract infections in children: a practical guide. Archives Of Pediatric Infectious Diseases.1;4(1),2016.

[2] S. M. Kareem, I.M. Al-Kadmy, S.S. Kazaal, A.N. Ali, S.N. Aziz, R.R. Makharita, A.M. Algammal, S. Al-Rejaie, T. Behl, G.E. Batiha and M.A. El-Mokhtar. Detection of gyra and parc mutations and prevalence of plasmid-mediated quinolone resistance genes in klebsiella pneumoniae. Infection and Drug Resistance. 14:555,2021.

[3] K.L. O'Brien, H.C. Baggett, W.A/ Brooks, D.R. Feikin, L.L. Hammitt, M.M. Higdon, S.R. Howie, M.D. Knoll, K.L. Kotloff, O.S. Levine and S.A. Madhi. Causes of severe pneumonia requiring hospital admission in children without HIV infection from Africa and Asia: the PERCH multi-country case-control study. The Lancet. 31;394(10200):757-79, 2019.

[4] S.V. Kumar, G.V. Kumar, J. Kandati, R.M. Pathapati, M. Buchineni and A. surveillance study of microbial pathogens and their antibiotic sensitivity of respiratory tract infections in a tertiary care hospital. Int J Curr Microbiol Appl Sci. 4(11):35-44, 2015.

[5] J.L. Mills, C.M. Flores, S. Reynolds, C. Wun,A. Calcutt, S.B. Baker, S. Murugappan, A.C. Depelsenaire, J. Dooley, P.V. Fahey and A.H. Forster. M-protein based vaccine induces immunogenicity and protection from Streptococcus pyogenes when delivered on a high-density microarray patch (HD-MAP). NPJ vaccines.7;5(1):1-1,2020.

[6] MG Caparon, DS Stephens, A Olsen, JR Scott. Role of M protein in adherence of group A streptococci. Infection and immunity. 59(5):1811-7,1991.

[7] F. Jamal, S. Pit, R. Facklam and B. Beall. New emm (M protein gene) sequences of group A streptococci isolated from Malaysian patients. Emerging infectious diseases.;5(1):182,1999.

[8] S.M. Kareem, I.M. Al-Kadmy, M.H. Al-Kaabi, S.N. Aziz and M. Ahmad. Acinetobacter baumannii virulence is enhanced by the combined presence of virulence factors genes phospholipase C (plcN) and elastase (lasB). Microbial pathogenesis. 1;110:568-72, 2017.

[9] S.S. Khazaal, I.M. Al-Kadmy and S.N. Aziz. Mechanism of pathogenesis in multidrug resistant

Acinetobacter baumannii isolated from intensive care unit. Gene Reports. 1;18:100557,2020.

[10] J.B. Dale, R.G. Washburn, M.B. Marques and M.R. Wessels. Hyaluronate capsule and surface M protein in resistance to opsonization of group A streptococci. Infection and immunity. May;64(5):1495-501,1996.

[11] S. Ibrahim, N. Al-Saryi, I. Al-Kadmy, and S.N. Aziz. Multidrug-resistant Acinetobacter baumannii as an emerging concern in hospitals. Molecular Biology Reports. 30:1-2, 2021

[12] K.E. Weinstein. Generation of Diversity During the Survival of Streptococcus pyogenes (Doctoral dissertation, Temple University. Libraries). 2010

[13] L. Baldassarri, R. Creti, S. Recchia, M. Imperi, B. Facinelli, E. Giovanetti, M. Pataracchia, G. Alfarone and G. Orefici. Therapeutic failures of antibiotics used to treat macrolide-susceptible Streptococcus pyogenes infections may be due to biofilm formation. Journal of clinical microbiology;44(8):2721-7,2006.

[14] Clinical and Laboratory Standards Institute (CLSI). Performance Standards for Antimicrobial Susceptibility Testing. 30th ed. CLSI supplement M100 (ISBN 978-1-68440-066-9 [Print]; SBN 978-1-68440-067-6 [Electronic]). Clinical and Laboratory Standards Institute, 950 West Valley Road, Suite 2500, Wayne, Pennsylvania 19087 USA, 2020.

[15] P.L. Ho, C. Cheung, G.C. Mak, W.S. Cindy, T.K. Ng, C.H. Cheung, T.L. Que, R. Lam, R.W. Lai, R.W. Yung and K.Y. Yuen.Molecular epidemiology and household transmission of community-associated methicillin-resistant Staphylococcus aureus in Hong Kong. Diagnostic microbiology and infectious disease. 1;57(2):145-51,2007.

[16] R. Abe, J.J. Ryan and R.J. Hodes. Clonal analysis of the Mls system. A reappraisal of polymorphism and allelism among Mlsa, Mlsc, and Mlsd. The Journal of experimental medicine.1;165(4):1113-29,1987.

[17] A.M. Timmer, S.A. Kristian, V. Datta, A. Jeng, C.M. Gillen, M.J. Walker, B. Beall and V. Nizet. Serum opacity factor promotes group A streptococcal epithelial cell invasion and virulence. Molecular microbiology.62(1):15-25,2006.

[18] SN Aziz, MF Al Marjani, AM Rheima, IM Al Kadmy. Antibacterial, antibiofilm, and antipersister cells formation of green synthesis silver nanoparticles and graphene nanosheets against Klebsiella pneumoniae. Reviews in Medical Microbiology. 2021 Apr 9.

[19] A. Gupta, G. Sarker and R. Pal. Risk correlates of acute respiratory infections in children under five years of age in slums of Bankura, West Bengal. Indian Journal of Child Health. 23;1(1):1-6, 2014.

[20] R. Virkki, T. Juven, H. Rikalainen, E. Svedström, J. Mertsola and O. Ruuskanen. Differentiation of bacterial and viral pneumonia in children. Thorax. 1;57(5):438-41,2002.

[21] S.N. Hamzah, M.N. Desa, A.S. Jasni,N.M. Taib,S.N.Masri and R.A.Hamat RA. Distribution of virulence genes and the molecular epidemiology of Streptococcus pyogenes clinical isolates by emm and multilocus sequence typing methods. 2020

[22] K Chawla, B Gurung, C Mukhopadhyay, I Bairy. Reporting emerging resistance of Streptococcus pneumoniae from India. Journal of global infectious diseases. 2010 Jan;2(1):10.

[23] D. Prabu and T. Menon. Genotyping of erythromycin resistant group C & G streptococci isolated in Chennai, south India. The Indian journal of medical research137(1):164,2013.

[24] L. Huber, S. Giguère, N.M. Slovis, S. Álvarez-Narváez, K.A/ Hart, M. Greiter, E.R. Morris and N.D. Cohen. The novel and transferable erm (51) gene confers macrolides, lincosamides and streptogramins B (MLSB) resistance to clonal Rhodococcus equi in the environment. Environmental microbiology. 22(7):2858-69,2020.

Cite this article

S. S. . Khazaal, S. K. . Al-Alak, and M. H. Z. . Talha, “Molecular Identification of Prevalent Streptococcus Pyogenes Serogroup Associated with Respiratory Tract Infections in Children”, *Al-Mustansiriyah Journal of Science*, vol. 33, no. 5, pp. 8–16, Feb. 2023.