



Original Research Article

Emerging Resistance to Erythromycin and Penicillin among *Streptococcus pyogenes* Isolates in Zagazig, Egypt

Salwa B Ibrahim¹, Rehab H. El-Sokkary^{1*}, Ahmed A. Elhewala²,
Mohammad Waheed El-Anwar³, Wael M. Awad⁴,
Abdelwahab M. Hamed⁴, and Inas I. Badawy⁵

¹Department of Medical Microbiology and Immunology – Faculty of Medicine Zagazig University, Egypt

²Department of Pediatrics – Faculty of Medicine Zagazig University, Egypt

³Department of Otorhinolaryngology, *Head and Neck Surgery*– Faculty of Medicine Zagazig University, Egypt

⁴Department of General Surgery – Faculty of Medicine Zagazig University, Egypt

⁵Specialist Medical Microbiology and Immunology- Ministry of health and population, Egypt

*Corresponding author

A B S T R A C T

Keywords

S.pyogenes, streptococci, infection control, antibiotics, erythromycin, Egypt, Penicillin, acute follicular tonsillitis, wound infection, resistance.

The rapidly growing problem of antibiotic resistant *Streptococcus pyogenes* is a major concern. The present study aimed to find out the incidence of *S. pyogenes* isolated from patients in our district, identify the susceptibility profile to commonly used antibiotics, determine MIC of isolates to penicillin and erythromycin and detect the macrolide resistance phenotypic pattern. A cross-sectional study with 180 enrolled patients was selected by systematic random sampling, presented with clinically suspected *S. pyogenes* infections (acute follicular tonsillitis, diabetic foot, infected surgical wounds & necrotizing fasciitis, without previous antibiotic therapy). Methods and Material: *S.pyogenes* were isolated & identified with antibiotic susceptibility testing. MIC determination was done for penicillin and erythromycin and determination of the macrolide resistance phenotypes. Statistical analysis: SPSS version 19 and EPI-INFO 6. Results: Three penicillin resistant strains (4%) and 16 erythromycin resistance strains (21.3%) were isolated. No vancomycin resistance, clindamycin resistance rate (10.7%). A rising Penicillin MIC (0.12 - 32) microgram/ml. Erythromycin MIC (1-256) microgram/ml. Phenotypic erythromycin resistance pattern showed M-phenotype; 11 strains (68.7%), 4 strains cMLS (25%) and 1 strain iMLS (6.3%). Conclusion: Incidence of erythromycin and penicillin resistance increased, restricted use of erythromycin and penicillin is advised to maintain required efficacy. Clindamycin is the drug of choice for penicillin resistant GAS infections in children while vancomycin is better to be saved for life-threatening conditions. Recommendations: Rationale use of penicillin is a must for not to lose this weapon against *S.pyogenes*. wide spread of resistance to erythromycin necessitates antibiotic-guided therapy.

Introduction

Streptococcus pyogenes (*S.pyogenes*) causes several infections as pharyngitis, pyoderma, cellulitis, necrotizing fasciitis, bacteremia and pneumonia, besides post-infectious

sequelae like acute rheumatic fever. Penicillin and its derivatives remain the drug of choice. However, in patients allergic to penicillin, alternatives as macrolide and quinolones are prescribed (Sharma et al.,

2010).

Increases in macrolide resistance have been reported. Moreover, the rapidly growing problem of antibiotic resistant *S.pyogenes* is increasing. Reports with rising minimum inhibitory concentration (MIC) or diminished susceptibility to penicillin have been published (Ray et al., 2010). Different resistance rates have existed according to geographical location and investigators (Bourbeau, 2003; Zhou et al., 2014). The main aim of this study to reveal incidence of *S.pyogenes* isolated from patients in our locality. And to identify susceptibility profile to commonly used antibiotics for the determine MIC of isolates to penicillin and erythromycin. Also to detect macrolide resistance phenotypic pattern.

Materials and methods

From May 2013 to December 2013, 180 patients were included in this cross sectional study. They were enrolled from patients attending at outpatient clinics of Otorhinolaryngolog, *Head and Neck Surgery*, General Surgery and Pediatric departments – Zagazig University hospitals-Zagazig-Egypt.

Selected patients were presented with infections clinically suspected to be caused by *S.pyogenes* with no previous antibiotic therapy as follows: 100 cases of acute follicular tonsillitis (AFT) (50 from pediatric outpatient clinics and 50 from Otorhinolaryngolog clinics), diabetic foot (30), infected surgical wounds (30) and necrotizing fasciitis (20). They were selected by systemic random sampling.

A thorough history was taken from all subjects. This study was approved by the Institutional Review Board (IRB) and informed written consent was obtained from

the enrolled subjects or parents of included children.

Identification of *S. pyogenes*: Samples collected included tonsillar pus, pus and wound exudates. Samples were transported to the lab in Amies transport medium (BBL, USA). Samples were processed and *S. pyogenes* identified by Gram stain, colony morphology on sheep blood agar containing trimethoprim-sulfamethoxazole, Catalase test and bacitracin sensitivity (Zone of inhibition >14 Cm) (Kotloff and Van Beneden, 2008).

Serologic identification: It was done by Pastorex strep A (Biorad), positive reaction appeared as red clumps on a green background and negative reaction appeared as uniform brown suspension.

Antibiotic susceptibility testing: It was done by disc diffusion method for the isolated (75) *Streptococcus pyogenes* strains according to the standard procedures of the CLSI guidelines. The following discs (Bioanalyse, Turkey) were used: Clindamycin [(DA) 2µg /disc], Erythromycin [(E) 15 µg/disc], Penicillin [(P) 10µg/dis], Vancomycin [(VA) 30µg /disc], Tetracycline [(TE) 30µg/disc] (CLSI, 2013).

MIC determination: E-test strips (AB-Biodisk) for both penicillin and erythromycin was used for all resistant isolates and tube dilution method for Benzathin benzyl penicillin (1200000 IU) from Acadima international, 1mg= 1307 IU Quality Control Standard Strain: *Streptococcus pneumoniae* ATCC49619. From the (Global Bioresource Center of American Type Culture Collection) (Soback, 2002).

Determination of the phenotypes of macrolide resistance

Erythromycin-resistant isolates were classified on the basis of their susceptibility

patterns as shown by double disk tests involving erythromycin (15 µg) and clindamycin (2 µg) disks (Becton Dickinson Microbiology Systems, Cockeysville, MD, USA). The 2 disks were placed 15 to 20 mm apart on Mueller-Hinton blood agar supplemented with 5% sheep blood, which had been inoculated with a swab dipped into a bacterial suspension with a turbidity equivalent to that of a 0.5 McFarland standard. Three phenotypes were assigned: M (erythromycin resistant and clindamycin susceptible), cMLSB (constitutive erythromycin and clindamycin resistant), and iMLSB (erythromycin resistant and clindamycin inducible). Blunting of the clindamycin inhibition zone near to the erythromycin disk indicated an iMLSB phenotype, whereas susceptibility to clindamycin with no blunting indicated the M phenotype (Seppala et al., 2005)

Statistical analysis: Data were collected, tabulated and analyzed using SPSS version 19 and EPI-INFO 6.

Result and Discussion

The included 180 patients were categorized into 3 age groups (Table 1). The cases were 100 female (55.6%) and 80 male (44.4%). Out of collected 180 clinical specimens, 75 isolates were identified as *S.pyogenes*. Results of antibiotic susceptibility test for those strains are shown in Table 2. Clinical presentation of isolated strains as regards penicillin susceptibility is illustrated in Table 3.

Results of MIC determination showed that, for the 3 penicillin resistant strains MIC range for penicillin was (8-32 ug/ml). The 3 strains were resistant to Benzathin penzyl penicillin G by tube dilution at concentration of 64 ug/ml. For the 16 erythromycin resistant isolates MIC range for

erythromycin was (1-256 ug/ml). Phenotypic pattern of macrolide resistance showed predominant M phenotype; 11 strains (68.7%), 4 strains cMLS (25%) and 1 strain iMLS (6.3%). The three penicillin resistant strains are demonstrated in Table 4.

S.pyogenes is a beta-hemolytic streptococcus of serological group A. It is one of the most important bacterial pathogens in humans. The management of *S.pyogenes* infection includes the use of penicillins, cephalosporins or macrolides for treatment however; recent studies have shown that changing strains of GAS resistant to these agents have emerged (Zhou et al., 2014).

In this study, we reevaluated in-vitro susceptibility of group A beta hemolytic streptococci in outpatient clinic of our hospitals. Antibiotic susceptibility pattern of isolated strains could help to set guidelines for empiric treatment of *S.pyogenes* in our district. Tetracycline is not recommended due to high resistance rate (32%). On the other hand vancomycin (100% susceptibility rate) is the most effective drug recommended as first line therapy in emergency and life threatening infections. Yet it may better be conserved to avoid spread of vancomycin resistance especially in mixed infections with MRSA and avoid consequent emergence of VRSA strains.

Penicillin showed excellent response with 90% susceptibility rate that permit to continue its use as the first line of treatment in *S.pyogenes* infections. However an alarm is rising about emerging resistance, 3 resistant strains (4%) were isolated in this study. Although low number, yet it is a very serious finding as it is the drug of choice in treatment of GAS infection.

A high rate of erythromycin resistance was

revealed in 16 strains representing 21.3% of isolated *S. pyogenes*. This was comparable to the results of previous studies (Ardanuy et al., 2010; Lynskey et al., 2011; Rubio-López

et al., 2012) higher than (Camara et al., 2013) and much lower than (Yang et al., 2013):

Table.1 Age distribution of enrolled cases

P	X ²	Age (years)			Diagnosis
		48-70 (n=41)	19-47 (n=52)	2-18 (N=87)	
<0.001**	36.83	9 (22.0%)	24 (46.2%)	67 (77.0%)	* AFT (n=100)
<0.001**	76.28	20 (48.8%)	—	—	Necrotizing Fasciitis (n=20)
<0.001**	50.8	6 (14.6%)	24 (46.2%)	—	Diabetic foot (n=30)
0.06	5.64	6 (14.6%)	4 (7.6%)	20 (23.0%)	Infected wound (n=30)

X² = Chi –Square test

Table.2 Antibiotic susceptibility pattern of *S. pyogenes* by Modified Kirby-Bauer method

Antibiotic agent (mg/disk)	Kirby-Bauer Susceptibility pattern in percentage (%)			Total
	Sensitive No. (%)	Intermediate No. (%)	Resistant No. (%)	
Penicillin (10µg)	68 (90.7%)	4 (5.3%)	3 (4.0%)	75
Erythromycin (15 µg)	33 (44.0%)	26 (34.7%)	16 (21.3%)	75
Clindamycin (2 µg)	67 (89.3%)	—	8 (10.7%)	75
Vancomycin (30 µg)	75 (100%)	—	—	75
Tetracycline (30 µg)	49 (65.3%)	2 (2.7%)	24 (32%)	75

Table.3 Association between susceptibility pattern for penicillin and erythromycin with clinical presentation

Susceptibility pattern	Clinical presentation				X ²	P
	AFT N=53	Necrotizing fasciitis N=3	Diabetic Foot N=7	Infected wound N=12		
Penicillin						
-Resistant	3 (5.7%)	—	—	—	3.294	0.771
-Intermediate	3 (5.7%)	—	1 (14.3%)	—		
-Sensitive	47 (88.6)	3 (100%)	6 (85.7%)	12 (100.0%)		
Erythromycin						
-Resistant	16 (30.2%)	—	—	—	9.622	0.142
-Intermediate	15 (28.3%)	1 (33.3%)	4 (57.1%)	6 (50.0%)		
-Sensitive	22 (41.55%)	2 (66.75%)	3 (42.9%)	6 (50.0%)		

X² = Chi –Square test

Table.4 Penicillin resistant isolated *Streptococcus pyogenes*

3	2	1	Strains Characteristics
10 years	10 years	12 years	Age
Resistant	Resistant	Resistant	Susceptibility to Erythromycin
M	M	iMLS	Erythromycin resistance pattern
sensitive	Sensitive	sensitive	Susceptibility to vancomycin and clindamycin
Resistant	Resistant	Resistant	Susceptibility to tetracycline

Rising allergy to Penicillin G with subsequent use of erythromycin as an alternative is implicated in high frequency of erythromycin resistance in many parts of the world (Villaseñor-Sierra et al., 2012). Thus erythromycin could be no more recommended as preferred choice in penicillin allergy. The revealed intermediate activity 5.3% in penicillin and 34.7% in erythromycin necessitates performing antibiotic susceptibility test before prescribing each.

The situation differs with clindamycin, it showed susceptibility rate comparable to that of penicillin (89.3%) but with higher resistance rate (10.7%). This makes it an accepted choice in respiratory infections caused by *S. pyogenes* in patients who are intolerant to other indicated antibiotics or who are infected with resistant organism and/or combined infections (e.g. diabetic foot). Clindamycin reduces the toxin producing effects of *S. pyogenes* and as such, may be particularly useful for treating necrotizing fasciitis.

In this study, we found out a rising Penicillin MIC with range 0.12 to 32

microgram/ml which is more than global

levels. European Society of clinical microbiology and infectious disease reported in 2013 that MIC of penicillin was < 0.25 microgram/ml against GA. Comparable results were revealed from different countries: India; 0.16 to 0.75 (Capoor et al., 2006) Mexico; 0.003 to 0.75 (Amábile-Cuevas et al., 2001), Japan 0.12 to 2 microgram/ml (Gawa et al., 2011). This highlights the importance of reconsidering patterns of penicillin susceptibility.

The three penicillin resistant strains were resistant also to erythromycin and to Benzathyn penzyl penicillin by tube dilution at concentration of 64 ug/ml. The phenotypic pattern of resistant strains was 2 M and 1 iMLS. This finding is in agreement with¹⁵ who stated that co-resistance to penicillin and erythromycin may be due to easy availability of antibiotics over the counter and their irrational use. However they were sensitive to clindamycin and vancomycin. This agreed with Friães et al in Portugal (Friães et al., 2012).

In this study, we found erythromycin MIC of 1 to 256 microgram/ml which agrees with

Bae et al. (2007). Phenotypic pattern of macrolide resistance showed predominant M phenotype; 11 strains (68.7%), 4 strains cMLS (25%) and 1 strain iMLS (6.3%). This results agreed with the results of previous researches (Rubio-López et al., 2012; Villaseñor-Sierra et al., 2012; Friães et al., 2012; Bae et al., 2007; Katz et al., 2003; Jacob et al., 2006). In general, high erythromycin resistance rates (90%) are associated with the MLSB phenotype, whereas the M phenotype is more frequent in countries with lower resistance (50%). It is well known that long-acting macrolides such as clarithromycin and azithromycin which ensure a low serum concentration of the antibiotic for a long period of time, are associated with the selection of resistant strains. The frequent use of such drugs in the Egyptian market as preferred treatment for GAS creates this selective pressure which could favor the spread of *S.pyogenes* clones carrying macrolide-resistant determinants, with consequent changes in macrolide resistance rates and phenotypes (Amer et al., 2008).

In conclusion, the incidence of erythromycin and penicillin resistance increased, restricted use of erythromycin and penicillin is advised to maintain required efficacy. Clindamycin is the drug of choice for penicillin resistant GAS infections in children while vancomycin is better to be saved for life threatening conditions.

Recommendations

Continuous local monitoring of resistance pattern of GAS to macrolides and other alternative drugs is recommended to avoid possible treatment failures. Further studies are needed to explore the genetic determinants of antibiotic resistance among *S. pyogenes* isolates.

Further studies are needed to evaluate effectiveness of long acting penicillin in both treating *S.pyogenes* infection and secondary prevention of rheumatic fever as there may be a need to increase dose to warrant more effective results.

References

- Amábile-Cuevas, C.F., Hermida-Escobedo, C., Vivar, R. 2001. Comparative in vitro activity of moxifloxacin by E-test against *Streptococcus pyogenes*. *Clin. Infect. Dis.*, 15(32): 30–32.
- Amer, F.A., Elbehedy, E.M., ElAhmady, M.E. 2008. *Streptococcus pneumoniae* in an Egyptian urban community: incidence of erythromycin resistance determinants and antibiotic susceptibility profile. *Asian Pac. J. Trop. Med.*, 1(94): 20–23.
- Ardanuy, C., Domenech, A., Rolo, D., Calatayud, L., Tubau, F., Ayats, J. et al., 2010. Molecular characterization of macrolide- and multidrug-resistant *Streptococcus pyogenes* isolated from adult patients in Barcelona, Spain (1993-2008). *J. Antimicrob. Chemother.*, 65(4): 634–643.
- Bae, S.Y., Kim, J.S., Kwon, J.A., Yoon, S.Y., Lim, C.S., Lee, K.N., et al. 2007. Phenotypes and genotypes of macrolide-resistant *Streptococcus pyogenes* isolated in Seoul, Korea. *J. Med. Microbiol.*, 56(Pt 2): 229–235.
- Bourbeau, P.P. 2003. Rule of the microbiology laboratory in diagnosis and management of pharyngitis. *J. Clin. Microbiol.*, 41: 3467–72.
- Camara, M., Dieng, A., boye, C.S.B. 2013. Antibiotic susceptibility of *Streptococcus Pyogenes* isolated from respiratory tract infections in Dakar, Senegal. *Microbiol. Insights*, 6: 71–75 .
- Capoor, M.R., Nair, D., Deb, M., Batra, K., Pushpa, A. 2006. Resistance to erythromycin and rising penicillin minimum inhibitory concentration in *Streptococcus pyogenes* in india. *J Infect. Dis.*, 95: 334–336.

- Clinical and Laboratory Standards Institute (CLSI), 2013. Disc diffusion supplemental tables Performance standards for antimicrobial susceptibility testing. M100-S23 (M02-A11).
- Friães, A., Pinto, F.R., Silva-Costa, C., Ramirez, M., Melo-Cristino, J. 2012. The Portugal group for the study of Streptococcal infections, Group A *Streptococci* clones associated with invasive infections and pharyngitis in Portugal present differences in *emm* types, superantigen gene content and antimicrobial resistance. *BMC Microbiol.*, 12: 280.
- Gawa, T., Terao, Y., Sakata, H. 2011. Epidemiological characterization of *Streptococcus pyogenes* isolated from patient with multiple onsets of pharyngitis. *FEMS Microbiol. let.*, 318: 143–156.
- Jacob, S.E., Lloyd, C.A.C., Menon, T. 2006. cMLS and M Phenotypes among *Streptococcus pyogenes* isolates in Chennai. *Indian J. Med. Microbiol.*, 224:147–148.
- Katz, K.C., McGeer, A.J., Duncan, C.L., Ashi-Sulaiman, A., Willey, B.M., Sarabia, A., et al., 2003. Emergence of macrolide resistance in throat culture isolates of group A *Streptococci* in Ontario, Canada, in 2001. *Antimicrob. Agents Chemother.*, 47: 2370–2372.
- Kotloff, K., Van Beneden, C. 2008. Standardization of epidemiologic protocols for surveillance of cute diseases caused by *Streptococcus pyogenes*: pharyngitis, impetigo, and invasive diseases, 1: 9–28.
- Lynskey, N.N., Lawrenson, R.A., Sirskandan, S. 2011. New understandings in *Streptococcus pyogenes*. *Curr. Opin. Infect. Dis.*, 24(3): 196–202.
- Ray, D., Sinha, S., Saha, S., Karmakar, S., Dutta, R.N., Bhattacharya, S. et al., 2010. A preliminary sentinel surveillance report on antibiotics resistance trend of *Streptococcus pyogenes* in Kolkata region, India. *Al Ameen J. Med. Sci.*, 3(2):146–151.
- Rubio-López, V., Valdezate, S., Álvarez, D., Villalón, P., Medina, M.J., CandSáez-Nieto, S.J.A. 2012. Molecular epidemiology, antimicrobial susceptibilities and resistance mechanisms of *Streptococcus pyogenes* isolates resistant to erythromycin and tetracycline in Spain (1994–2006). *BMC Microbiol.*, 12: 215.
- Seppala, H., Nissinen, A., Yu, Q., Huovinen, P. 2005. Three different phenotypes of erythromycin- resistance *Streptococcus pyogenes* in Finland. *J. Antimicrob. Chemother.*, 32(6): 885–891.
- Sharma, Y., Vishwanath, S., Bairy, I. 2010. Biotype and antibiotic resistance pattern of group A streptococci. *Indian J Pathol Microbiol.*, 53: 187–188.
- Soback, S. 2002. Susceptibility testing, adeterminant of successful therapy. Poultry medicin publication. Aavailable in <http://www.poultrymed.com/>. Accessed [February 27, 2014].
- Villaseñor-Sierra, A., Katahira, E., Jaramillo-Valdivia, A.N., Barajas-GarcíaMde, L., Bryant, A., Morfín-Otero, R. et al., 2012. Phenotypes and genotypes of erythromycin-resistant *Streptococcus pyogenes* strains isolated from invasive and non-invasive infections from Mexico and the USA during 1999–2010. *Int. J. Infect. Dis.*, 16(3): 178–181.
- Yang, P., Peng, X., Zhang, D., Shuangsheng, Wu., Liu, Y., Cui, S., et al., 2013. Characteristics of Group A *Streptococcus* strains circulating during scarlet fever epidemic, Beijing, China, 2011. *Emerg. Infect. Dis.*, 19(6): 909–915. Available in <http://wwwnc.cdc.gov/eid/article/19/6/12>.
- Zhou, W., Jiang, Y.M., Wang, H.J., Kuang, L.H., Hu, Z.Q., Shi, H., et al. 2014. Erythromycin-resistant genes in group A β -haemolytic *Streptococci* in Chengdu, Southwestern China. *Indian J. Med. Microbiol.*, 32: 290–3.