Original article

Streptococcus peumoniae in an Egyptian urban community: incidence of erythromycin-resistance determinants and antibiotic susceptibility profile

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Abstract

Objectives: To determine the incidence of resistance of *Streptococcus* (*Strep*). *pneumoniae* isolated in our locality to erythromycin, to screen for the two resistance determinants *erm*(B) and *mef*(A) genes, and to identify the susceptibility profile to commonly used antibiotics. **Methods**: Samples were collected from patients attending the Outpatient Department of Zagazig University Hospital, Zagazig, Egypt, between February 2006 and March 2007. *Strep. pneumoniae* was identified by conventional procedures. Susceptibilities to erythromycin and 15 antibiotics were identified by disc diffusion method, as outlined by CLSI. E-test was used for MIC determination of erythromycin. *erm*(B) and *mef*(A) genes were detected by PCR. **Results**: Eighty-one *Strep. pneumoniae* strains were identified. Fifty- one of them (63 %) were erythromycin-resistant, and *mef*(A) gene was the predominant resistance determinant. Vancomycin, imipenem and gatifloxacin had the best activity against the isolates, whereas tetracycline had the least. Forty-two (51.85%) out of the 81 *Strep. pneumoniae* strains were multidrug-resistant. **Conclusions**: High incidence of resistance to erythromycin and multiple antimicrobials existed. *mef*(A) was the principal erythromycin-resistance gene.

Keywords: *Strep. pneumoniae*; Erythromycin; *erm*(B) gene; *mef*(A) gene; Antibiotic resistance; Egypt

INTRODUCTION

Macrolide resistance in *Strep. pneumoniae* has increased during the 1990s to the extent that over 70% of clinical isolates are now resistant in some communities [1]. Many macrolide-resistant *Pneumococci* are also resistant to β- lactams, and non β- lactam compounds limiting treatment alternatives.

Four mechanisms of macrolide resistance have been described in *Strep. pneumoniae*. They are ribosomal methylation encoded by the *erm*(B) gene [2], macrolide efflux encoded by the *mef*(A) gene [3], and mutations within rRNA and ribosomal protein[4]. More recently, resistance in clinical isolates harboring the *erm*(A) subclass *erm*(TR) gene has been described [5].

The *erm*(B) gene is associated with high-level resistance to macrolides, lincosamides, and streptogramin B (MLS_B phenotype), while the *mef*(A) gene is associated with low-level resistance to 14- and 15-membered-ring macrolides (M phenotype). The distribution of these two genes varies between locations.

The objectives of this work were to determine the incidence of resistance of *Strep. pneumoniae* isolated in our locality to erythromycin, to screen for the two resistance determinants *erm*(B) and *mef*(A) genes, and to identify the susceptibility profile to commonly used antibiotics.

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MATERIALS AND METHODS

Between February 2006 and March 2007, 433 clinical samples were collected from patients attending the Outpatient Department of Zagazig University Hospitals, Zagazig, Egypt. They were presenting with infections suspected to be caused by *Strep. pneumoniae*.

Samples collected included postnasal discharge from patients with sinusitis, middle ear fluid from patients with acute otitis media, cerebrospinal fluid (CSF) from patients with meningitis, conjunctival swabs from patients with conjunctivitis and sputum and blood samples from patients with pneumonia. Samples were transported to the laboratory in Amies medium (BBL, NJ, USA), while blood samples were directly inoculated into Signal Blood Culture bottles (Oxoid, Basingstoke, UK). Samples were processed and *Strep. pneumoniae* identified by Gram stain, colonial morphology, optochin sensitivity, bile solubility and mouse inoculation [6].

Susceptibility to eryhtromycin as well as commonly used antibiotics was carried out by disc diffusion methods, according to the standard procedures of the CLSI guidelines [7]. The following discs (Oxoid, Basingstoke, UK), were used: erythromycin (30 μg), penicillin (10 μg), ampicillin (30 μg), amoxycillin-clavulanate (30 μg), cefazolin (30 μg), cefotaxime (30 μg), cefuroxime (30 μg), ciprofloxacin (5 μg), clindamycin (30 μg), gatifloxacin (5 μg), imipenem (10 μg), ofloxacin (5 μg), oxytetracycline (30 μg), piperacillin (75 μg), rifampicin (30 μg) and vancomycin (30 μg). The minimum inhibitory concentration of erythromycin to *Strep. pneumoniae* was determined using E-test strips (AB Biodisk, Solna, Sweden).

The *mef*(A) and *erm*(B) genes were detected after PCR amplification, as previously described [8]. The primers were *erm*(B) (upstream, 5′-GAA AAG GTA CTC AAC CAA ATA-3′; downstream, 5′-GTA ACG GTA CTT AAA TTG TTT AC-3′) and *mef*(A) (upstream, 5′-AGT ATC ATT AAT CAC TAG TGC-3′; downstream, 5′-TTC TTC TGG TAC TAA AAG TGG-3′) [9], (VBC Genomics, Vienna, Austria).

RESULTS

Eighty-one strains of *Strep. pneumoniae* were isolated. Fifty-one strains (62.96%) were resistant to erythromycin. The MIC range, MIC_{50} and MIC_{90} of erythromycin were 0.032- 128 μg/mL, 16 μg/mL and 128 μg/mL, respectively.

mef(A) gene was detected in all strains; alone in 32 (39.51%), while together with *erm*(B) in 49 (60.49%) of them (Figure).

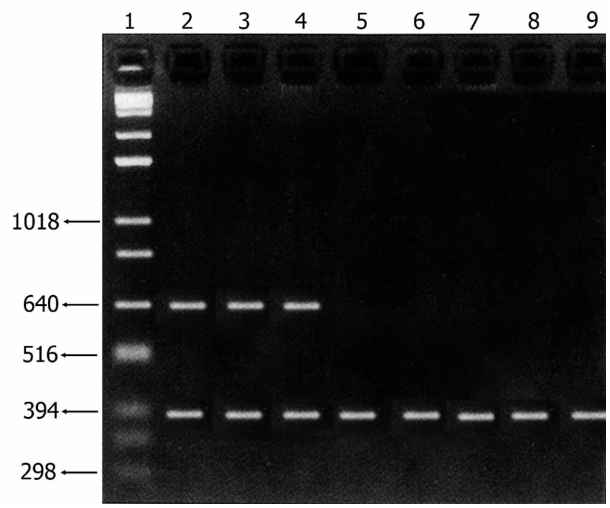

Figure: PCR analysis of erythromycin-resistance genes. Lane 1: MW marker. Lanes 5-9: *mef*(A) gene only (nearly at 348 bp). Lanes 2-4: Both *mef*(A) gene (nearly at 348 bp) and *erm*(B) gene (nearly at 640 bp).

All isolates were sensitive to vancomycin, imipenem and gatifloxacin. The susceptibilities to the rest of antibiotics in a descending order were: amoxicillin-clavulanate (75 strains, 92.59%), rifampicin (70 strains, 86.41%), ampicillin (66 strains, 81.48%), cefotaxime (66 strains, 81.48%), ciprofloxacin (61 strains, 75.31%), cefuroxime (57 strains, 70.37%), clindamycin (57 strains, 70.37%), cefazolin (54 strains, 66.67%), piperacillin (49 strains, 60.49%), penicillin (24 strains, 29.62%), and tetracyclines (15 strains, 18.51%). Forty-two (51.85%) out of the 81 *Strep. pneumoniae* isolates were multidrug-resistant.

DISSCUSION

We encountered an elevated rate of erythromycin resistance among our *Strep. pneumoniae* isolates (62.96%), which is comparable to rates reported from France (56%) [10], higher than rates from Germany[11], Spain [12] and the eastern south-central

parts of USA [13] (14.1%, 34.5% and 47%, respectively), and lower than rates from Asian countries (more than 70% in Vietnam, Hong Kong, Taiwan, South Korea and China [1]).

Resistance to erythromycin displayed by our isolates was of high-level. This may be due to the presence in the two-thirds of the srains of *erm*(B) gene, known to be associated with high-level erythromycin resistance [9].

Whereas *erm*(B) was the predominant gene encoding erythromycin resistance in south Africa, most Asian countries and almost all European countries, *mef*(A) was reported to be the main determinant in Greece, Canada, USA, Hong Kong, Singapore, Thailand, Malaysia and the present study. Combination of the two genes also existed in our work as has been reported by others, which adds an evidence to previous conclusions that combination does not necessarily have distinct geographic distribution but can be identified from diverse locations [8, 9, 12, 14-21].

Susceptibility pattern of *Strep. pneumoniae* in this work is providing guidelines for empiric prescription of antibiotics in our locality. Tetracycline is not recommended. Its poor activity may be explained by the high incidence of *erm*(B) gene amongst our isolates, known to be harbored along with tetracycline resistance gene *tet*(M) on the conjugative transposon *Tn*1545 [22]. Moreover, erythromycin and penicillin are no more indicated as first line therapy. In spite of the high incidence of resistance to penicillin (70%), the majority of our strains were sensitive to ampicillin (83.3%). This can be due to the possibility of a point mutation affecting the gene responsible for production of low MW, PBPs. Such mutation affects the action of penicillin only and interferes with the transfer of resistance as a single genetic event [23]. Gatifloxacin is the most active quinolone derivative, followed by ciprofloxacin then ofloxacin. This may be an outcome of the more recent introduction of gatifloxacin in the Egyptian pharmaceutical market and its high price. The intermediate activity of piperacillin, 1st and 2nd generation cephalosporins and clindamycin necessitates sensitivity testing before prescription. On the other hand, the better degree of effectiveness of cefotaxime, amoxicillin-clavulanate and rifampicin are permitting a wider scale of use. Imipenem and vancomycin are the most effective agents, so they could be used as first line therapy in life- threatening infections before the sensitivity test result is available (which is also applicable to gatifloxacin).

In the present work 42 (51.85%) out of the 81 *Strep. pneumoniae* strains were multidrug resistant. All these strains were also resistant to penicillin. As has been concluded a relationship exists between resistance to penicillin and resistance to other antimicrobial drug classes [13]. It is worth mentioning that multidrug resistance can make it hard to treat infections caused by *Strep. pneumoniae*. Therefore, disease prevention should be a priority. This can be achieved by increasing the use of the 23-valent pneumococcal polysaccharide vaccine among high-risk adults and older persons [24], by use of the new 7-valent conjugate vaccine in children [25], and by implementation of improved and novel hygiene practices.

In conclusion, high rate of erythromycin resistance is evident among our isolates and *mef*(A) gene is the main resistance determinant. Resistance to other antibiotics also exists which will influence therapeutic decisions.

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