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Prevalence of gyrA and parE mutations in clinical isolates of Streptococcus pneumoniae with decreased susceptibilities to different Fluoroquinolones

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Abstract

Introduction: Streptococcus pneumoniae is a major Gram-positive pathogen responsible for pneumonia, bactermia, otitis media, and meningitis leading to considerable morbidity and mortality among children and elderly individuals. The primary goals of antibiotic treatment of respiratory tract infections are clinical efficacy of treatment, pathogen eradication, and prevention of resistance development. Resistance to fluoroquinolones in S. pneumoniae arises in a stepwise fashion and results from alterations in the target binding site due to the acquisition of spontaneous mutations in the quinolone resistance-determining regions (QRDRs) of the topoisomerase IV and DNA gyrase genes. Although mutations usually occur in the QRDRs of parC and gyrA, a role for mutations in the parE subunit in low-level resistance has been reported.

Aim of the work: The aim of this study was to determine the prevalence of fluoroquinolone resistance Streptococcus pneumoniae (FQRSP) and to examine the genetic relatedness of pneumococcal isolates with parE and gyrA genes mutations in different specimens in Sohag University Hospital.

Patients and Methods: This study was prospectively conducted over a period of 24 months between October 2015 and September 2017, at Sohag university hospital. During the study period, 78 patients hospitalized for a syndrome consistent with a diagnosis of community acquired pneumonia (CAP) included in this study with a mean age of 34.5 years (range, 2 to 67), 60% of whom were males. A CAP syndrome was defined as a newly recognized pulmonary infiltrate together with 2 of the following findings: subjective fever or documented temperature 37.4 °C, increased cough, sputum production, or shortness of breath, pleuritic chest pain, confusion, rales, leukocytosis, (according to age) **(1)**. Patients who had taken antibiotic treatment within 3 days prior to initial visit were excluded from this study.

Results: Our study illustrate the role of mutation in the gyrA&parE genes and the effect of mutations in the both genes in fluoroquinolone resistance among S. pneumoniae isolates.

Conclusion: The present study provide an opportunity to view the predominant mutations conferring reduced susceptibility to FQs in clinical pneumococcal isolates. There is a strong relationship between these mutations and decrese susceptibility to the most fameous FQs to some extent, although this varies between strains and for each drug.

Key words: GyrA, ParE, Streptococcus pneumonia, Fluoroquinolones.

Introduction

Streptococcus pneumoniae is a major Gram-positive pathogen responsible for

pneumonia, bactermia, otitis media, and meningitis leading to considerable morbidity and mortality among children and elderly individuals. The primary goals of antibiotic treatment of respiratory tract infections are clinical efficacy of treatment, pathogen eradication, and prevention ofresistance development. Penicillin, a β-lactam antibiotic, has long been the mainstay against pneumococcal infections, but the worldwide spread of antibiotic-resistant clones over the past decades has impaired its usefulness for dealing with S. pneumoniae infections. The rates of resistance against β-lactams and macrolides among S. pneumoniae isolates have translated into anincreased usage of fluoroquinolone antibiotics in the treatment of respiratory diseases (2).
Since their introductionintoclinical use the fluoroquinolones havehada major impact on the treatment of moderate-to-severe infections.Their broad spectrum ofactivity,clinical utility, availability in bothoraland parenteralforms,andfavorable pharmacokinetic propertieshascontributed to theirextensiveworldwideuse.
However, in recentyearsbacterial resistance to thefluoroquinoloneshasbecome a majorconcern. Fluoroquinolones are part of aclassof synthetic broad-spectrumantibioticsthat inhibit DNA synthesis inbacteriaby targeting DNA gyrase (GyrAand-B subunits) and topoisomeraseIV(ParCand -E subunits), two enzymesthatare vital for DNA supercoiling and chromosome segregation,respectively(3).
The rise ingram-positivepathogen resistance in recent yearshasprompted the pharmaceutical industrytodevelop fluoroquinolones withgreateractivity against these rapidlychangingpathogens.Structural modifications tothebasicfluoroquinolone nucleus havegivenriseto several new generations of compounds. With eachnewgeneration the potency againstmanygram-positive pathogens, including S.pneumoniae,hasimproved.Although the worldwide prevalence offluoroquinolone-resistantS. pneumoniae remains low in relation to β-lactam resistance (<1%), the dissemination of successful resistant clones has nonetheless increased the prevalence in some countries (4).
Resistance to fluoroquinolones in S. pneumoniae arises in a stepwise fashion and results from alterations in the target binding site due to the acquisition of spontaneous mutations in the quinolone resistance-determining regions (QRDRs) of the topoisomerase IV and DNA gyrase genes. Although mutations usually occur in the QRDRs of parE and gyrA, a role for mutations in the parE and gyrAsubunits in low-level resistance has been reported (5).
Inappropriate use of any antibiotic can contribute to the emergence of resistance to that and related agents. So much work is needed to identify optimal strategies to prevent the emergence and spread of resistant pneumococcal strains in long-term care facilities, including potential use of pneumococcal conjugate vaccines, antimicrobial stewardship, and infection control interventions to interrupt transmission (6).

Aim of the work

The aim of this study was to determine the prevalence of fluoroquinolone resistance Streptococcus pneumoniae (FQRSP) and to examine the genetic relatedness of pneumococcal isolates with parE and gyrA genes mutations in different specimens.

Patients and Methods:

Patients: This study was prospectively conducted over a period of 24 months between October 2015 and September 2017, at Sohag university hospital. During the study period, 78 patients hospitalized for a syndrome consistent with a diagnosis of CAP included in this study with a mean age of 34.5 years (range, 2 to 67), 60% of whom were males.

A CAP syndrome was defined as a newly recognized pulmonary infiltrate together with 2 of the following findings: subjective fever or documented temperature 37.4 °C, increased cough, sputum production, or shortness of breath, pleuritic chest pain, confusion, rales, leukocytosis, (according to age) **(1)**. Patients who had taken antibiotic treatment within 3 days prior to initial visit were excluded from thisstudy.

Methods

Specific investigations

Clinical specimens and clinical laboratory work.

Fresh sputum samples were collected soon after collection of data from patients (75 specimens). Representative sputum originating from the lower respiratory tract was defined as that containing > 25 granulocytes and < 10 epithelial cells per low power field (lpf: total magnification:×100)**(1)**.

Bronchoalveolar lavage (BAL) (3 specimens) as diagnostic techniques were used according to the clinical judgment of the physician in charge for some neonates.

Microbiologic Evaluation Culture method

Isolates were incubated in plates with increased CO2 (5-10%) in order to enhance the development of hemolytic zones of the pathogenic Streptococci and incubated for 18-24 hours. In all cases, growth requires a source of catalase (e.g. blood) to neutralize the large amount of H2O2 produced by the bacteria.

By gram stain isolates appear as lancet-shaped, Gram-positive diplococci or chains of cocci.

The identification of bacteria in our samples was completed by the VITEK® 2 Compact System. As a commercialand standard system, its accuracy has been strictly evaluated.

Susceptibility product information Intended Use

The VITEK® 2 Antimicrobial Susceptibility Tests (AST) are intended for use with the VITEK® 2 Systems for the automated quantitative or qualitative susceptibility testing of isolated colonies for most clinically significant aerobic Gram-negative bacilli, Staphylococcus spp., Enterococcus spp., Streptococcus spp., S. pneumoniae, and yeast.

Summary and Explanation of the Test

Susceptibility testing is indicated for any organism that contributes to an infectious processwarranting antimicrobial chemotherapy. Susceptibility tests are most often indicated when the causative organism is thought to belong to a species capable of exhibiting resistance to commonly used agents. Isolated colonies of each type of organism that may play a pathogenic role are selected from an agar plate and tested for susceptibility. These tests are then examined and the Minimum Inhibitory Concentration (MIC) is determined. The MIC obtained using a dilution test may tell the physician the concentration of an antimicrobial agent needed at the site of infection to inhibit the infecting organism. MICs have traditionally been determined using antimicrobial concentrations derived from Serial twofold dilutions of MIC is then determined from the lowest

concentration that exhibits inhibition of growth. An interpretive criterion (Susceptible, Intermediate, or Resistant) can then be assigned to MIC results to aid in the direction of therapy. For some antimicrobials (e.g., high-level gentamicin, high-level streptomycin) a qualitative result is generated. The standard and reference procedures are based on susceptibility tests requiring 16 to 24 hours of incubation for bacteria and 24 to 48 hours for yeast. Various manufacturers have now developed automated procedures designed togenerateresults more rapidly by using shortenedrim. Then the DNA was centrifuged at incubation times. Laboratories worldwide14000 rpm for 30 seconds. This wash use either variations of the standardstepwasrepeated.
reference procedure or a commercially available product to determine the MICs of
≥ 25 ul DNA Elution Buffer wasinfectious organisms.

Polymerase chain reaction(PCR):-

Simple PCR was performed for all strains to detectfluoroquinolone resistance Streptococcus pneumoniae (FQRSP) and to examine the genetic relatedness of pneumococcal isolates with parE and gyrA genes mutations.

i) Bacterial DNApurification.

By the use of DNA Clean& Concentrator
™-25 (catalog No D4033).
Before starting: 26 ml 95% ethanol was added to the 6 ml DNA Wash Buffer concentrate.

Protocol:- (according to manufacture instruction)

1- The DNA bands were exiced from the agarose gel with a sterile razor plate and placed in a 1.5 ml microcentrifuge tube, 5 volumes of DNA Binding Buffer were added to each volume of DNA samples placed at 50 °C for 10 minutes . Then mixed by pulse-vortexing for 15 seconds every 2-3 minutes till the agarose completely dissolved.
2- Then the DNA (including the precipitate) was carefully added to a provided Zymo-Spin™ Column in a 2 ml Collection Tube without wetting the rim. Then the cap was closed to avoid aerosol formation duringcentrifugation.
3- The DNA was centrifuged at 14000 rpm for 30 seconds. Then the Zymo-Spin™ Column was placed in a clean 2 ml collection tube and the tube contating the filterate wasdiscarded.
4- 200 ul DNA Wash Buffer was added to the column without wettingthe
added directly to the column matrix without wetting the rim and incubate at room temperature for one minute. The Zymo-Spin™ Column was placed in a clean 1.5 ml microcentrifuge tube then centrifuged at 14000 rpm for 30 seconds to elute theDNA.
Ultra-Pure DNA was ready to use for the next sequencing step.

ii) DNAsequencing

Sequencing reactio were prepared with ABI Prism® BigDye Terminator Cycle Sequencing Ready Reaction Kit using conditions descriped by Zhanel et al. **(8)** with ABI 377 automated sequencer (PE Applied Biosystems, mississauga, ON).

Sequence analysis

DNA sequences were analyzed with DNASIS 2.6 Sequence Analysis Programs (Hitachi Software Engineering Co., Ltd., San Francisco, Calif.) against 1 of the 2 identical sequenced pneumococcal strains in the database (NC_008533 Streptococcus pneumoniae D39 and AE007317). D39 is a
historically important serotype 2 strain that was used in experiments

by Avery and coworkers to demonstrate that DNA is the genetic material.

Results

During the period from October 2015 and September 2017 our study was carried out in the Clinical Pathology Department, faculty of medicine, Sohag University Hospital, 78 participants included in our study, the majority of the isolates were isolated from males, 49 (62.8%) male and 29 (37.2%) female, aged ranged from 1 to 64 years with mean age 22.2±20.8. Inflammatory biomarkers differentiating viral from bacterial infections have been evaluated in our study to support clinical diagnosis. The majority of our study group had high ESR values 65 (83.3%), and positive CRP 64 (82.1%). Also in our study, it was observed that WBCs was high in 63/78 (82.1%) of cases. S. pneumoniae was isolated from 78 patients included in this study. The resistance percentages of all strains to tested antibiotics were as follows: 91% of isolates in our study were resistant to Ampicillin, 5.1% were intermediate and 3.8% were susceptible. Regarding Cefaclor 83.3% were resistant, 7.7% were intermediate and 9% were susceptible. Erythromycin was resistant in 82.1% of isolates, intermediate in 10.3%, and susceptible in 7.7%. Regarding Imipenem 10.3% of isolates were resistant. Tetracycline was resistant in 71.8% of isolates. Clarithromycin was resistant in only 6.4%, also 10.3% of our isolates were resistant to ceftriaxone. Trimethoprim/ sulfamethoxazole was resistant in 9% of ourisolates.

Break points of antibiotics "Ampicillin" ≥ 2 R & 0.12 – 1 I & ≤ 0.06 S , "Cefaclor" > 16 R & 8 – 16 I & ≤ 4 S, "Erythromycin" ≥ 1 R & 0.5 I & ≤ 0.25 S, "Imipenem" ≥ 1 R & 0.5 I & ≤ 0.25 S "Tetracyclin" ≥ 8 R & 4 I & ≤ 2 S, "Clarithromycin" ≥ 2 R & 1 I & ≤ 0.5 S, "Ceftriaxone" ≥ 2 R & 1 I & ≤ 0.5 S and "Trimethoprim-Sulfamethaxzole " ≥ 4 R & 1 – 2 I & ≤ 0.5S.

Among 78 isolates 37 (47.4%) of S. pneumonia isolates were Fluroquinolones susceptible 12 (15.4%) were with variable susceptibility and 29 (37.2%) were Fluroquinolones resistant.

The MICs of Ciprofloxacin, Levofloxacin, Gatifloxacin and Moxifloxacin were measured and results were as follow, 44.9% of S. pneumonia isolates were resistant to ciprofloxacin, 11.5% were intermediate and 43.6% were sensitive. Regarding levofloxacin 42.3% of isolates were resistant, 9% were intermediate, and 48.7% were sensitive. Over forty six (46.1%) of our isolates were resistant to Gatfloxacin, 10.3% were intermediate, and 43.6% were sensitive. Regarding Moxifloxacin 46.2% of our isolates were resistant, 7.6% were intermediate, and 46.2% were sensitive (Table 1). Break points of fluroquinolones group "Ciprofloxacin" ≥ 4 R & 2 I & ≤ 1 S and "Levofloxacin" ≥ 8 R & 4 I & ≤ 2 S and "Gatfloxacin" ≥ 4 R & 2 I & ≤ 1 S "Moxifloxacin" ≥ 4 R & 2 I & ≤ 1S.

Table (1) Fluroquinolones susceptibility of S. pneumoniae.

Variable	MIC(ug/dl)	no (%)
Ciprofloxacin (1^{st}generation Fluoroquinolone)	Resistant ≥ 4	35(44.9%)
	Intermediate 2	9(11.5%)
	Susceptible ≤ 1	34 (43.6%)
Levofloxacin (2^{nd}generation Fluoroquinolone)	Intermediate4	7(9%)
	Susceptible ≤ 2	38(48.7%)
Gatfloxacin (3^{rd}generation Fluoroquinolone)	Resistant ≥ 4	36(46.1%)
	Intermediate2	8(10.3%)
	Susceptible ≤ 1	34 (43.6%)
Moxifloxacin (4^{th}generation Fluoroquinolone)	Resistant ≥ 4	36(46.2%)
	Intermediate 2	6(7.6%)
	Susceptible ≤ 1	36(46.2%)

I. Sequencing of the Quinolone Resistance-Determining(QRDRs).

Of the 41 quinolone resistant and intermediate isolates isolates, 9 (22.0%) had no substitutions in the QRDRs of either GyrA or ParE, 19 (46.3%) had a QRDR GyrA substitution, while 16 (39.0%) had QRDRs substitutions in ParE.

The specific substitutions observed in in **GyrA**were Ser81Phe, Ser81Tyr and Glu85Lys. The specific substitutions observed in **ParE**were Asp435Asn and Glu407Lys. The percent of isolates with each of the aforementioned substitutions is presented in table (2).

Table (2) Percent and types of the 41 Fluroquinolones-resistant S. pneumoniae isolates with resistance-associated QRDR substitutions.

Variable		no (%)
GyrA	Glu85Lys	1 (2.4%)
	Ser81Phe	13 (31.7%)
	Ser81Tyr	5 (12.2%)
ParE	Asp435Asn	6 (14.6%)
	Glu407Lys	2(4.9%)

Table 3 show percentage of genes mutations in each antibiotic of Fluroquinolones-resistant S. pneumoniae

Variable	Gyr A	Par E
Ciprofloxacin	18(56.3%)	8(25%)
Levofloxacin	18(56.3%)	8(25%)
Gatfloxacin	18(56.3%)	8(25%)
Moxifloxacin	18(56.3%)	8(25%)

As shown in table 4, at Ciprofloxacin MIC 2, 4, 8 and 16, 75%, 55.6%, 33.3% and 30% had no substitution in **Gyr A**, only 10% of ciprofloxacin resistantand intermediate isolates had Glu85Lys substitution at MIC 16. Also at MIC 2, 4, 8 and 16, there were 25%, 33.1%, 66.7% and 30% Ser81Phe substitution respectively. At MIC 4 and 16 there were 11.1% and 30% had Ser81Tyr substitution.

RegardingParE,atCiprofloxacinMIC2,4,8and16,50%,66.7%,77.85and 10%hadnosubstitutionatParE.25%,22.2%,22.2%and10%hadAsp435Asn substitution. Only 25% and 11.1% at MIC 1 and 4 had Glu407Lys substitution.

Table (4) Ciprofloxacin MICs and substitutions observed in GyrA and ParE in ciprofloxacin-resistant and intermediate S. pneumoniae isolates.

Variable(MIC)	2	4	8	16
GyrA				
No substitution	3 (75%)	5 (55.6%)	3 (33.3%)	3 (30%)
Glu85Lys	0 (0%)	0 (0%)	0 (0%)	1(10%)
Ser81Phe	1 (25%)	3 (33.1%)	6 (66.7%)	3 (30%)
Ser81Tyr	0 (0%)	1 (11.1%)	0 (0%)	3 (30%)
Par E				
No substitution	2 (50%)	6 (66.7%)	7 (77.8%)	9 (90%)
Asp435Asn	1 (25%)	2 (22.2%)	2 (22.2%)	1 (10%)
Glu407Lys	1 (25%)	1 (11.1%)	0 (0%)	0 (0%)

As shown in table (5) at Levofloxacin MIC 4, 8 and 16, 100%, 53.8% and 21.4% had no substitution in **Gyr A**, only 7.7% of levofloxacin resistant and intermediate isolates had Glu85Lys substitution at MIC 8. Also at MIC 8 and 16, there were 30.8% and 57.2% Ser81Phe substitution respectively. At MIC 8 and 16 there were 7.7% and 21.4% had Ser81Tyrsubstitution.

Regarding Par E, at Levofloxacin MIC 4, 8 and 16, 50%, 69.2% and 85.7% had no substitution at Par E. 25%, 23.1% and 14.3% had Asp435Asn substitution. Only 25% and 7.7% at MIC 4 and 8 had Glu407Lys substitution.

Table (5) Levofloxacin MICs and substitutions observed in GyrA and ParE in ciprofloxacin-resistant and intermediate S. pneumoniae isolates.

Variable(MIC)	4	8	16
GyrA			
No substitution	4 (100%)	7 (53.8%)	3 (21.4%)
Glu85Lys	0 (0%)	1 (7.7%)	0 (0%)
Ser81Phe	0 (0%)	4 (30.8%)	8 (57.2%)
Ser81Tyr	0 (0%)	1 (7.7%)	3 (21.4%)
Par E			
No substitution	2 (50%)	9 (69.2%)	12 (85.7%)
Asp435Asn	1 (25%)	3 (23.1%)	2 (14.3%)
Glu407Lys	1 (25%)	1 (7.7%)	0 (0%)

As shown in table 6, at Gatfloxacin MIC 2, 4, 8 and 16, 75%, 66.6%, 41.7% and 11.1% had no substitution in **Gyr A**, only 11.1% of gatfloxacin resistant and intermediate isolates had Glu85Lys substitution at MIC 16. Also at MIC 2, 4, 8 and 16, there were 25%, 16.7%, 50% and 44.5% Ser81Phe substitution respectively. At
MIC 4, 8 and 16 there were 16.7%, 8.3% and 33.3% had Ser81Tyr substitution.

Regarding Par E, at Gatfloxacin MIC 2, 4, 8 and 16, 75%, 83.3%, 66.7%and
77.8% had no substitution at Par E. 16.7%, 33.3% and 11.1% had Asp435Asn
substitution at MIC 4, 8 and 16. Only 25% and 11.1% at MIC 2 and 16 had Glu407Lyssubstitution.

Table (6) Gatfloxacin MICs and substitutions observed in GyrA and ParE in ciprofloxacin-resistant and intermediate S. pneumoniae isolates

Variable(MIC)	2	4	8	16
GyrA				
No substitution	3 (75%)	4 (66.6%)	5 (41.7%)	1 (11.1%)
Glu85Lys	0 (0%)	0 (0%)	0 (0%)	1 (11.1%)
Ser81Phe	1 (25%)	1 (16.7%)	6 (50%)	4 (44.5%)
Ser81Tyr	0 (0%)	1 (16.7%)	1 (8.3%)	3 (33. 3%)
Par E				
No substitution	3 (75%)	5 (83.3%)	8 (66.7%)	7 (77.8%)
Asp435Asn	0 (0%)	1 (16.7%)	4 (33.3%)	1 (11.1%)
Glu407Lys	1 (25%)	0 (0%)	0 (0%)	1 (11.1%)

As shown in table 7, at Moxifloxacin MIC 2, 4, 8 and 16, 50%, 55.6%, 40% and 25% had no substitution in **Gyr A**, only 11.1% of moxifloxacin resistant and intermediate isolates had Glu85Lys substitution at MIC 4. Also at MIC 2, 4, 8 and16, therewere25%,33.3%,30%and62.5%Ser81Phesubstitutionrespectively.AtMIC 2, 8 and 16 there were 25%, 30% and 12.5% had Ser81Tyr substitution.

Regarding Par E, at Moxifloxacin MIC 2, 4, 8 and 16, 75%, 66.7%, 80%and 75% had no substitution at Par E. 25%, 22.2%, 10% and 25% had Asp435Asn substitution at MIC 2, 4, 8 and 16. Only 11.1% and 10% at MIC 4 and 8 had Glu407Lys substitution.

Table (7) Moxifloxacin MICs and substitutions observed in GyrA and ParE in ciprofloxacin-resistant and intermediate S. pneumoniae isolates

Variable(MIC)	2	4	8	16
GyrA				
No substitution	2 (50%)	5 (55.6%)	4 (40%)	2 (25%)
Glu85Lys	0 (0%)	1 (11.1%)	0 (0%)	0 (0%)
Ser81Phe	1 (25%)	3 (33.3%)	3 (30%)	5 (62.5%)
Ser81Tyr	1 (25%)	0 (0%)	3 (30%)	1 (12.5%)
Par E				
No substitution	3 (75%)	6 (66.7%)	8 (80%)	6 (75%)
Asp435Asn	1 (25%)	2 (22.2%)	1 (10%)	2 (25%)
Glu407Lys	0 (0%)	1 (11.1%)	1 (10%)	0 (0%)

Discussion

Mean age of our study group was 22.2±20.8, and ranged from 1 to64, our study included 49 (62.8%)males and 29 (37.2%) females. Majority of ourstudygrouphadhighESR,WBCs and positive CRP. In study of Kargar et al. [(11)] 16 (35.33%) were maleand 29 (64.45%) were female.

Ninty one (91%) of isolates in our study were resistant to ampicillin,5.1% were intermediate and 3.8% were susceptible. Regarding cefaclor 83.3% were resistant, 7.7% were intermediate and 9% were susceptible. Erythromycin was resistant in 82.1% of isolates, intermediate in 10.3%, and susceptible in 7.7%. Regarding imipenem 10.3% of isolates were resistant. Tetracycline was resistant in 71.8% of isolates. Clarithromycin was resistant in only 6.4%, also 10.3% of our isolates were resistant to the antibiotics ceftriaxone.Trimethoprim/sulfamethoxazoleresistant in 9% of our isolates.

The MICs of Ciprofloxacin, Levofloxacin, Gatifloxacin and Moxifloxacin were measured in this study and we found that, 44.9% of S. pneumonia isolates were resistant to ciprofloxacin, 11.5%were intermediate and 43.6% were sensitive. Regarding levofloxacin 42.3% of isolates were resistant, 9% were intermediate, and 48.7% were sensitive. Over forty six (46.1%) of our isolates were resistant to Gatfloxacin, 10.3% were intermediate, and 43.6% were sensitive. Regarding Moxifloxacin 46.2% of our isolates were resistant, 7.6% were intermediate, and 46.2% were sensitive. Also in study of Kargar et al. [(11)] the resistance percentages of all strains to tested antibiotics were as follows: ciprofloxacin73.33%, Ofloxacin 53.33%, Norfloxacin 48.89%, and levofloxacin 42.22%. The highest resistance was observed in patients in the age group of 31-40 years [(11)].

Regarding genetic substitution, we found that at ciprofloxacin MIC 2, 4, 8

and16,75%,55.6%,33.3%and30% had no substitution in Gyr A, only 10% of ciprofloxacin resistant and intermediate

isolates had Glu85Lys substitution at MIC 16. Also at MIC 2, 4,8and16,therewere25%,33.1%, 66.7% and 30% Ser81Phe substitution respectively. At MIC 4 and 16 there were 11.1% and 30% had Ser81Tyr substitution. On the other hand, at Par E, at ciprofloxacin MIC 2, 4, 8 and 16, 50%, 66.7%, 77.85 and 10% hadno substitution at Par E. 25%,22.2%, 22.2% and 10% had Asp435Asn substitution. Only 25% and 11.1%at MIC 1 and 4 had Glu407Lys substitution.

Similar to our results, in studies of Bast et al. [12], Broskey et al. [13], Brueggemann et al. [14] the GyrA substitutions observed most often were at positions Ser81 (Phe or Tyr) (54% of ciprofloxacin-resistant isolates) and Glu85 (Gly or Lys) (10% of ciprofloxacin-resistant

is

olates). Overall, the most common genotype observed was Ser79Phe (ParC) and Ser81Phe (GyrA) (35% of ciprofloxacinresistant isolates). Also in study of Korzheva et al. **[15]** substitutions at Ser81 in GyrA are believed to be the most commonly observed substitutions as these positions interact with the fluoroquinolone in the ternary complex.

Regarding levofloxacin, we found that at levofloxacin MIC 4, 8 and 16, 100%, 53.8% and 21.4% had no substitution in Gyr A, only 7.7% of levofloxacin resistant and intermediate isolates had Glu85Lys substitution at MIC8.AlsoatMIC8and16,there were 30.8% and 57.2% Ser81Phe substitution respectively. At MIC 8 and 16 there were 7.7% and 21.4% had Ser81Tyr substitution. In Par E, at levofloxacin MIC 4, 8 and 16,50%, 69.2% and 85.7% had nosubstitution at Par E. 25%, 23.1% and 14.3% had Asp435Asn substitution. Only 25% and 7.7% at MIC 4 and 8 had Glu407Lys substitution. On the other hand, previous studies reported that between 59% and 71% of isolates with levofloxacin MICs of 2 pg/mL had QRDR substitutions in GyrA**[16, 17]**. Few studies have evaluated isolates with levofloxacin MICs of 1pg/mL. The MIC 90 of levofloxacin for S. pneumoniae is 1 pg/mL. The use of this MIC provides the greatest number of isolates for study and is a sensitive measure of shifts over time**[8]**.

Regarding gatfloxacin, we found thatatgatfloxacinMIC2,4,8and16, 75%, 66.6%, 41.7% and 11.1% had no substitution in Gyr A, only 11.1% of gatfloxacin resistant and intermediate isolates had Glu85Lys substitution at MIC16.AlsoatMIC2,4,8and16, there were 25%, 16.7%, 50% and 44.5% Ser81Phe substitution respectively.AtMIC4,8and16there were 16.7%, 8.3% and 33.3% had Ser81Tyr substitution. In Par E, at gatfloxacinMIC2,4,8and16,75%, 83.3%, 66.7% and 77.8% had no substitution at Par E. 16.7%, 33.3% and11.1% had Asp435Asn substitution at MIC 4, 8 and 16. Only 25%and 11.1% at MIC 2 and 16 had Glu407Lyssubstitution.

Regarding moxifloxacin, we found thatatmoxifloxacinMIC2,4,8 and 16, 50%, 55.6%, 40% and 25% had no substitution in Gyr A, only 11.1% of moxifloxacin resistant and intermediate isolates had Glu85Lys substitution at MIC 4. Also at MIC 2, 4,8and16,therewere25%,33.3%, 30% and 62.5% Ser81Phe substitution

respectively.AtMIC2,8and16 there were 25%, 30% and 12.5% had Ser81Tyr substitution. In Par E, at moxifloxacin MIC 2, 4, 8 and 16,75%, 66.7%, 80% and 75% hadno substitution at Par E. 25%,22.2%, 10% and 25% had Asp435Asn substitution at MIC 2, 4, 8 and 16. Only 11.1% and 10% at MIC 4 and 8 had Glu407Lys substitution.

In study of Kargar et al. [11] investigated the prevalence of mutations in the parE and gyrA genes and their role in the development of quinolone resistance. Their findings imply that only mutations in gyrA gene were resistant to ciprofloxacin, susceptible to ofloxacin, and semisusceptible to levofloxacin. However, the isolates that had simultaneous mutations in both genes were completely resistant to ofloxacin and levofloxacin [18]. Also, there are various opinions about the parE gene and its role in the development of resistance to quinolones; according to research by Kawamura-Sato et al. [19] in Japan and Credito et al. [20] in the United States, isolates that had parE gene mutations, along with mutations in the gyrA gene, had higher resistance to ciprofloxacin, ofloxacin, norfloxacin, and lorfloxacin than mutants that did not have mutations in the parE gene. Findings of Kargar etal. [11]iscontrarytotheresultsofIpetal. [21], which showed that the strains possess mutations in the parE gene are susceptible to quinolones and have no difference from the wild strains. In contrast to results of Ip et al. [21], for the first time in Iran, Kargar et al. [11] also illustrated that there is a significant correlation between mutations in the parE gene and resistance to norfloxacin, as strains that had mutations in the gyrA gene along with mutations in the parE gene showed higher levels of resistance to thisantibiotic.

Sierra et al. [22] recently correlated mutagenic potency of the fluoroquinolones to likelihood of mutant selection. They found levofloxacin and moxifloxacin to be less mutagenic than ciprofloxacin and gemifloxacin and resistant mutants to be selected most commonly by ciprofloxacin followed by gemifloxacin, moxifloxacin and levofloxacin [22]. In study of Brino et al. [23] most isolates had mutations at conventional sites in gyrA (codons for S81 and E85), the amino acid position most frequently reported to be associated with resistance of pneumococci to this class of agents. In addition, 3 of these 16 isolates had multiple mutations that included sites in parE. Fass et al. [24] found that only the MICs of levofloxacin and ofloxacin were increased with the introduction of this mutation intoparE.

Furtherore, no single mutants of gyrA were found in the isolates included in the study of Fukuda and Hiramatsu. [25] and single mutants of gyrA have been detected among strains selected in vitro onnewer

Conclusion

There is a strong relationship between these mutations and decrese susceptibility to the most fameous FQs to some extent,

Recommandations

We recommend:

1. Close attention to monitor fluoroquinolone susceptibility patterns and the association of multidrug resistance with fluoroquinolone resistance in isolates ofS.pneumoniae.
2. The increased prescription of fluoroquinolones as first-line therapy for common infections such as respiratory tract infection will facilitate the emergence of resistance to this class of compounds and promote the

emergence of multidrug-resistant strains and, therefore, should be discouraged as it will undermine the efficacy of fluoroquinolones to treat more-seriousinfections.

3. Continued surveillance of respiratory tract isolates and other pathogens is important, and appropriate clinical use of fluoroquinolones is imperative as they become more widelyprescribed.
4. Further studies in larger numbers of patients are necessary to establish the role of genesubstitution

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fluoroquinolones, such as sparfloxacin or gatifloxacin**(25, 26)**, but are rarely reported for clinical isolates. This may reflect the relatively recent use of newer fluoroquinolones that select for gyrA mutations first. Also, isolates with a single mutation in gyrA may be overlooked if the MIC of the fluoroquinolone agent(s) used to screen for resistance is unchanged or only modestlyincreased. although this varies between strains and for eachdrug. in (QRDRs) in S. pneumonia isolates and resistance toFluoroquinlones.

The maintenance of such surveillance is valuable in the preparation of future therapy guidelines and could lead to new therapeutic strategies for FQ-resistant S.Pneumoniae.

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