Exposure to Aminoglycosides from Flu Vaccines and Susceptibility to Gentamicin Among Escherichia coli Strains in Urinary Isolates from Asthmatic Patients

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Objective: To evaluate the antimicrobial resistance pattern of *Escherichia coli*, an important pathogen associated with urinary tract infections, in asthmatic adult patients previously repeatedly exposed to residual amounts of aminoglycosides from flu vaccines.
Material and methods: We determined the antibiotic susceptibility of *Escherichia coli* strains isolated from the specimens of adult asthmatic subjects with urinary tract infections, hospitalized in an allergy university clinic in Bucharest. All patients were enrolled in the previous six months, are known with persistent asthma treated with controller medication, and received seasonal influenza vaccination annually at least the last two years, with or without a previous A/H1N1 pandemic flu vaccination, with vaccines containing traces of aminoglycosides. A control group included adult patients with a positive history of adverse drug reactions not vaccinated for influenza and not treated with aminoglycosides in the last two years. Antimicrobial susceptibility testing was performed by Kirby-Bauer disc diffusion method.
Results: We found that overall the gentamicin susceptibility rate is high in analysed samples from the enrolled patients (94.82%). Gentamicin resistance is very low in both groups, with evidently no statistical increase in resistance to this antibiotic in the *Escherichia coli* isolated from the urine of asthmatic patients previously parenteral exposed to influenza vaccines containing residual amounts of neomycin or gentamicin.
Conclusion: The annually administration of injectable flu vaccines containing aminoglycosides in order to protect high-risk groups against the variable influenza virus seems not to influence gentamicin susceptibility pattern of *Escherichia coli* strains.

Keywords: aminoglycosides, influenza vaccines, *Escherichia coli* susceptibility

Introduction

Incorrect use of antibiotics increases antimicrobial resistance. Many million courses of antibiotics are used each year for viral conditions that do not benefit from these drugs. Besides being applied in human therapy, antibiotics are extensively used for animal farming, aquaculture, and for agricultural purposes. An important consequence of antibiotic residual presence in natural products and environmental pollution by antibiotics may be the selection of resistant bacteria [1,2]. Moreover, exposure to residual gentamicin from antibiotic-loaded polymethylmethacrylate beads sometimes used for arthroplasty may potentially induce antibiotic resistance [3].

Repeated parenteral exposure to residual amounts of aminoglycosides from many influenza vaccines may at least theoretically influence the susceptibility to gentamicin among bacterial strains. In the context of above mentioned information, the study objective is to evaluate the antimicrobial resistance pattern of *Escherichia coli*, an important pathogen associated with urinary tract infections, in asthmatic adult patients previously repeatedly exposed to residual amounts of neomycin or gentamicin from flu vaccines.

Material and methods

We determined the antibiotic susceptibility of *Escherichia coli* strains isolated from adult asthmatic subjects with urinary tract infections, hospitalized in the allergy university clinic Hospital "*Nicolae Malaxa*" in Bucharest.

All patients were enrolled in the previous six months, are known with persistent asthma treated with *controller* medication (inhaled glucocorticosteroids alone or in combination with inhaled long-acting selective beta$_2$-agonits, with or without an oral leukotriene modifier), and received seasonal influenza vaccination annually at least the last two years, with or without a previous A/H1N1 pandemic flu vaccination, with vaccines containing residual amounts of aminoglycosides (Cantgrip, Fluarix, Vaxigrip).

A control group included adult patients with a positive history of adverse drug reactions not vaccinated and not treated with aminoglycosides in the last two years.

Data susceptibility on *Escherichia coli* strains isolated from these patients with community-acquired urinary tract infections were collected. Isolates were analysed by standard methods and antimicrobial susceptibility testing was performed by Kirby-Bauer disc diffusion method, with standard controls. This Kirby-Bauer antibiotic testing (KB testing or disk diffusion antibiotic sensitivity testing) is a test which uses antibiotic-impregnated wafers to test whether particular bacteria are susceptible to specific antibiotics. Each urine isolate was tested for *in vitro* susceptibility to amoxicillin-clavulanate, cefuroxime, gentamicin, ciprofloxacin and cotrimoxazol.

For statistical analyses a χ^2 test was performed to detect differences in uropathogen susceptibility between groups. A p value of ≤ 0.05 was considered significant.

Results

Eleven adult asthmatic patients enrolled were optimally controlled with inhaled asthma *controller* regimens with ciclesonide pressurized metered-dose inhaler (Alvesco 80 inhaler) or dry powder inhaler combinations, such as budesonide-formoterol (Symbicort Turbuhaler 160/4.5 mcg) or salmeterol-fluticasone (Seretide Diskus 50/250 mcg sau 50/500 mcg), with or without oral montelukast (Singulair 10 mg). No asthmatic patient or control received antibiotics in the last month. Asthmatic subjects received seasonal influenza vaccination annually at least the last two years (Fluarix or Vaxigrip), the majority being also administered also the A/H1N1 pandemic flu vaccine (Cantgrip), all these vaccines containing residual amounts of aminoglycosides.

A total of fifty-eight *Escherichia coli* strains were examined, from which twenty-eight from adult asthmatic patients anually exposed to influenza vaccines containing small amounts of neomycin or gentamicin sulfate, and thirty from control subjects not previously exposed to aminoglycosides.

We found that overall the gentamicin susceptibility rate is high in analysed samples from the enrolled patients (94.82%). Gentamicin resistance is very low in both groups (Table I), with evidently no statistical increase in resistance to this antibiotic in the Escherichia coli isolated from the urine of asthmatic patients previously repeatedly exposed to residual amounts of aminoglycosides from influenza vaccines. The rate of resistance to ciprofloxacin is very low in gentamicin-susceptible isolates (1.81%) (Table I).

These data obviously reveal that repeated intramuscular exposure to residual amounts of neomycin or gentamicin sulfate from flu vaccines does not negatively influence the susceptibility to gentamicin among bacterial strains.

Discussions

Aminoglycosides are still a very important class of antibacterials due to their broad antimicrobial spectrum, rapid bactericidal action and ability to act synergistically with other antibiotics, such as beta-lactams [4], especially in the treatment of severe illness caused by a variety of pathogens, including Gram-negative bacteria.

These drugs act by causing translational errors and by inhibiting translocation, the target sites including ribosomal domains in which the accuracy of codon-anticodon is assessed. There are several mechanisms involved in the induction of the resistance to aminoglycosides: decreased intracellular accumulation of the antibiotic (by altering the outer membrane permeability, less inner membrane transport, or active efflux), enzymatic modification of the drug (primarily through N-acetylation, O-nucleotidylation, or O-phosphorylation), modification of the target by mutation in ribosomal proteins or in 16S rRNA, and trapping of the drug [5].

Table I. Susceptibility to gentamicin of Escherichia coli strains

Group (n)	Vaccinated asthmatic patients (28)	Control subjects (30)
Gender	85.71% females	83.33% females
Gentamicin resistance	3.57%	6.66%

The mechanism of resistance to aminoglycosides based on methylation of their target, 16S rRNA, was until recently described only in antibiotic producing microorganisms. Equivalent methyltransferases have recently also been identified among numerous clinical Gram-negative pathogenic isolates [6].

It is generally accepted that two 16S rRNA methylations, m^7G1405 and m^1A1408, confer resistance to overlapping sets of aminoglycoside antibiotics [4].

The *arm*A (aminoglycoside resistance methyltransferase) gene was mentioned as a gene that confers resistance to 4,6-disubstituted deoxystreptamines (kanamycin, amikacin, isepamicin, gentamicin, netilmicin, sisomicin, and tobramycin), but not against 4,5-disubstituted deoxystreptamines (lividomycin, neomycin, paromomycin, ribostamycin) [5].

In a more recent attempt to describe a gene conferring multiple aminoglycoside resistance in *Escherichia coli*, bovine-origin isolates were tested for resistance phenotype using a disk diffusion assay and for resistance genotype using a DNA microarray. Isolate with amikacin and gentamicin resistance with no corresponding gene detection yielded a 1056 bp DNA sequence with closest homologues for its inferred protein sequence among a family of 16S rRNA methyltransferase enzymes which confer high-level aminoglycoside resistance [7].

In order to investigate the molecular aspects of aminoglycoside resistance among *Escherichia coli* isolates from humans and food-producing animals, another recent study revealed that more than 75% of the gentamicin-resistant isolates from human and animal sources were found to possess the aacC2 gene, a gentamicin resistance determinant involved in a hospital epidemic of multiply resistant members of the family *Enterobacteriaceae*. In some isolates, the aacC2 gene was encoded on large transferable plasmids of multiple incompatibility groups (IncF, IncI1 and IncN). This research showed that human and animal isolates share the same pool of resistance genes [8].

According to international guidelines, patients with asthma should be advised to receive a seasonal influenza vaccination annually and when vaccination of the general population is advised, such as the A/H1N1 pandemic influenza vaccination. Respiratory influenza infections are more serious in patients with asthma, and such infections can often lead to pneumonia and acute respiratory disease. Influenza vaccines are one of the most effective ways to protect people from contracting illness during influenza epidemics and pandemics [9,10,11].

Seasonal influenza vaccination is indicated for active immunization for the prevention of disease caused by influenza virus subtypes A and type B contained in the vaccine. Some influenza virus vaccines for intramuscular injection, prepared from viruses propagated in embryonated chicken eggs, may contain residual amounts of gentamicin sulfate ≤ 0.15 mcg per 0.5 mL i.m. dose, from the manufacturing process [12]. Other inactivated split virion influenza vaccines may contain traces of neomycin (< 20 pg) per 0.5 mL i.m. dose [13]. In the last year, pandemic influenza vaccination was performed in conjunction with the seasonal flu vaccine. Some influenza A/H1N1 2009 monovalent inactivated vaccines contain traces of neomycin sulfate (0.2 pg-2.5 mcg) per 0.5 mL i.m. dose [14,15]. Other pandemic split inactivated influenza vaccines contain residual amounts of gentamicin sulfate (≤ 0.15 mcg per 0.5 mL i.m. dose).

Repeated administration of flu vaccines is needed due to annual reformulation of influenza vaccines. Each year, three strains are chosen for selection in that year's influenza vaccination by the WHO Global Influenza Surveillance Network.

Because antibiotic residues due to low doses added to cattle, poultry and swine feed in order to promote growth may be a cause of bacterial resistance [16], we evaluated the antimicrobial resistance pattern in *Escherichia coli* in adult patients previously repeatedly exposed to residual amounts of aminoglycosides from influenza vaccines. Our data reveal that repeated intramuscular exposure to residual amounts of neomycin or gentamicin from flu vaccines does not influence the susceptibility to gentamicin among uropathogen bacterial strains. To out knowledge, no other studies has yet proved that the presence of antibiotic residues in vaccines has directly been the cause of an increase in antibioresistances.

Conclusions

The annual administration in high-risk asthmatic group of injectable influenza vaccines, containing residues of aminoglycosides, seems not to influence gentamicin susceptibility pattern of *Escherichia coli* strains in these subjects.

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