

Antibiotic resistance in *Escherichia coli* isolated from animal and municipal wastewater treatment plants in Slovakia.

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Abstract

Waste water treatment plants used mechanical, chemical and biological means for discharging safe wastewater into recipient. Repeated exposure of bacteria to antimicrobial agents and access of bacteria to increasingly large pools of antimicrobial resistance genes in mixed bacterial populations are the primary driving forces for emerging antimicrobial resistance.

The aim of the study was to evaluate the occurrence of antibiotic resistance and ESBLs in *E. coli* isolated from municipal and animal wastewaters.

Introduction

Fecal coliforms are excreted in the feces by humans and animals and ultimately reach wastewater treatment plants. Using mechanical, chemical and biological means, wastewater treatment plants treat wastewater for discharge into the environment. Treatment of wastewaters involves agitation, aeration and mechanical movement which is associated with splashing and popping of bubbles and release of aerosols laden with microorganisms (termed bioaerosols) into the air.

Antibiotics are used to improve public health and quality of life worldwide. However, incomplete metabolism in humans has resulted in release of large quantities of pharmaceutical drugs into municipal wastewater treatment plants [3].

Recent studies revealed presence of low concentrations of antibiotics in wastewater treatment plant effluents and surface waters. The prevalence of antibiotics in municipal wastewater and surface waters can lead to the development of antibiotic resistant bacteria due to a long-term exposure to low concentrations of antibiotics in the ng /L to µg/L range [2].

Antibiotic resistance is primarily caused by antibiotic use, which has led to initiatives to restrict antibiotic prescriptions and curtail antibiotic use in agriculture.

Material and Methods

During the year 2011 we collected wastewater samples from waste water treatment plant effluent after UV disinfection. The samples were filtered and differential bacterial diagnosis was carried out on relevant nutrient media.

Sixty isolates of *E. coli* from municipal and animal wastewater treatment plants were analysed for the presence of ESBLs and high level fluoroquinolone resistance. Antibiotic resistance was determined phenotypically according to CLSI, M31-A3 [1] using ampicillin, ampicillin and sulbactam, ceftiofur, ceftriaxon, ceftazidime, ceftazidime and clavulanic acid, gentamicin, streptomycin, neomycin, spectinomycin, nalidixic acid, enrofloxacin, ciprofloxacin, chloramphenicol, florfenicol, tetracycline and cotrimoxazol. The presence of CTX-M groups, CMY-2, integron 1, Tn3 and phylogenetic analysis was determined by PCR. Potential clonal relatedness of the studied isolates was determined by Maldi tof analysis.

Results

Environmental *E. coli* showed high occurrence of betalactam resistance: ampicillin 96%, ampicillin with sulbactam 20%, ceftiofur 73%, cefquinome 18% and ceftriaxon 41 %. Ertapenem resistance was not found. The ESBL phenotype was present in 30 strains, multiresistance in 23 strains and high level fluoroquinolone resistance (CIP \geq 4mg/L) in 25 strains. We were able to confirm the presence of CTX-

M1 group genes in 13 isolates and CMY-2 gene in 30 isolates from all ESBLs phenotypes. The CTX-M1 and CMY-2 genes were associated in all isolates with a class 1 integron and Tn3. Phylogenetic analysis revealed the majority of the pathogen B2 and D groups. However, only one municipal and one animal isolate showed clear clonal relatedness, with distance level 100 (Maldi tof biotyping).

ATB	R	MIC XG
Ampicillin	96.67%	97
Ampicillin+sulbactam	20%	11.4
Ceftriaxon	41.67	11.7
Ceftiofur	73.33%	7.1
Ceftazidime	5%	2.1
Cefquinome	18.33%	8.5
Gentamicin	20%	1
Streptomycin	60%	36.8
Neomycin	21.67%	6.3
Spectinomycin	30%	35.1
Nalidix acid	66.67%	50.2
Ciprofloxacin	41.67%	0.7
Enrofloxacin	45%	1.7
Tetracycline	78.33%	15.6
Chloramfenicol	38.33%	12
Florfenicol	30%	11.7
Cotrimoxazol	60%	38.1

Table 1. MIC and percentage of resistance in E.coli from waste water treatment plant

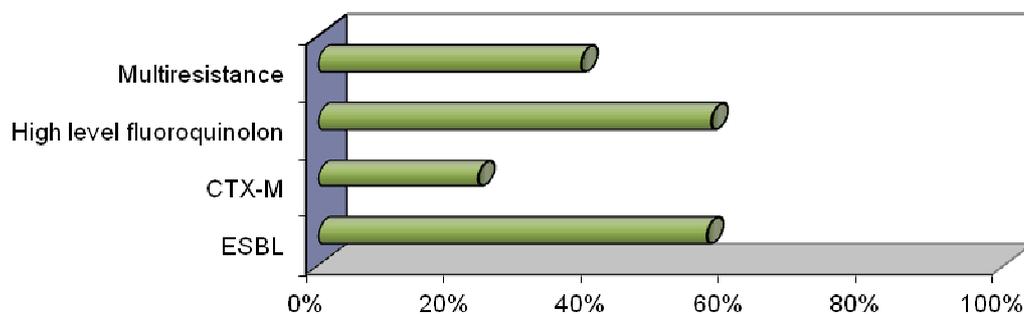


Figure 1. Resistance mechanisms in E.coli

Conclusions and prospectives

1. CTX-M1 group and CMY-2 genes associated with integron 1 and Tn3 were frequently detected in environmental *E.coli*.

2. Ertapenem resistance was not found.

3. The majority of strains belonged to the B2 and D phylogenetic groups. Only one municipal and one animal isolate showed clear clonal relatedness (Maldi tof).

Resistant microorganisms that exist in wastewater treatment plant environment can pose risk to animals and people particularly through exposure to infectious antibiotic-resistant pathogens or commensals and related potential mortality and failure of therapy. Antimicrobial-resistant pathogens tend to be more virulent than their susceptible counterparts, causing more prolonged or severe illnesses [4].

Acknowledgements.

This study was supported by the grant No. APVV- 0009-10 of the Slovak Research and Development Agency

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