УДК 628.35

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ANTIBIOTIC RESISTANT ESCHERICHIA COLI AND STAPHYLOCOCCUS AUREUS IN CZECH WASTEWATER TREATMENT PLANT

1. INTRODUCTION

Presence of pathogenic microorganisms and especially of resistant pathogenic microorganisms in environment, mainly in wastewater is becoming a real threat for human. The most common nosocomial pathogens are methicillin- and vancomycin-resistant *Staphylococcus aureus* strains, multiresistant *Enterococcus* spp. and *Pseudomonas aeruginosa*, and also some members of *Enterobacteriaceae* (Vincent, 2003; Jeljaszewicz *et al*., 2000). Essentially, the presence of *Staphylococcus aureus* and *Escherichia coli* and their resistant strains in the environment represents a great threat for human health (Hawkey, 2008).

Escherichia coli include nonpathogenic strains and also pathogenic strains, including enterotoxigenic (ETEC), enteropathogenic (EPEC), enteroagregative (EAggEC), enterohemorrhagic (EHEC), enteroinvasive (EIEC) and verotoxin or Shiga-toxin producing (VTEC, STEC) strains responsible for different serious illnesses (Mhone *et al*., 2011). *Staphylococcus aureus* is a common cause of variety of nosocomial infections, including folliculitis, endocarditis, osteomyelitis, septic arthritis, metastatic abscess formations and postoperative septicaemia (Edwards *et al.*, 2011).

Fortunately, not all infections are serious, but antibiotic therapy may be ordinarily required. Frequent antibiotic prescription results in increasing prevalence of resistant microbial strains (Gardner *et al.*, 2011). The treatment of bacterial infections is complicated by the development of its single or multi-drug resistant strains. Moreover, the treatment of imunosuppressed and weakened people is not always sufficient. The resistance of *S. aureus* to ciprofloxacin, penicillin and especially to methicillin has been discussed deeply (Lowy, 2003). In particular, resistance to vancomycin, last available antibiotic to treat resistant *S. aureus* infections (Howden *et al.*, 2011), has also emerged.

Antibiotic concentration in wastewater is also increasing due to their frequent misuse (prescription for viral infection) and ending up in wastewater treatment plant (WWTP). Due to their incomplete biological degradation their original residues or metabolites may be excreted through urine and faeces into the sewerage. Considering their antibacterial effects, they are not totally disposed during biological clearing processes in WWTPs. As a result sub-inhibitive concentrations of antibiotics in wastewater has occurred (Karaolia *et al.*, 2014). Another factor contributing to dissemination of resistance is horizontal transfer of resistant isolates genes to antibiotic sensitive microbial species occurring in wastewater resulting in development of mutation in responses to antibiotic presence (Baquero *et al*., 2008).

Aim of this work was to monitor and compare prevalence of antibiotic resistant *Escherichia coli* and *Staphylococcus aureus* in different samples of wastewater (WWTP influent, effluent, hospital and almhouse) and stabilized sludge from different Czech cities.

2. MATERIALS AND METHODS

Samples of influent, effluent water and stabilized sludge were taken from different Czech WWTPs (A – Zubří, B – Valašské Meziříčí) and also in wastewaters from hospital (B – Valašské Meziříčí, C – Rožnov pod Radhoštem) and almshouse (B – Valašské Meziříčí) in these towns. The cultivation dilution method on selective growth media was used to determine the density of selected microbial groups in wastewater and stabilized sludge. The counts of total coliforms and *E. coli* were determined on chromogenic Chromocult agar (Merck, Darmstadt, Germany) and total staphylococci and coagulase-positive *S. aureus* were determined on Baird-Parker agar (Sigma-Aldrich, St. Louis, USA). The microbial profile of effluent waters was analysed after concentration of 10 ml water through GH-PolyPro hydrophilic membranes with pores of 0.45 μm (VWR, Wien, Austria). Resistant strains of *E. coli* or *S. aureus* were determined on the same agar with an antibiotic addition (AMP – ampicillin, CIP – ciprofloxacin, CEF – cefoxitin, ERY – erythromycin, GEN – gentamycin, CHL – chloramphenicol, 4C – tetracycline, VAN – vancomycin, PEN – penicillin, MET – methicillin; all Sigma-Aldrich, St. Louis, USA). Antibiotic resistance was detected according to European (EUCAST) as well as to US (CLSI) resistance breakpoints.

3. RESULTS

In our work we were interested in the prevalence of resistant *S. aureus* and *E. coli* in wastewater (inflow and outflow from WWTPs) and sludge in two different Czech cities (A, B) and also in wastewaters from hospital and almshouse in the studied towns. The representation of total coliforms and staphylococci, and also of *E. coli* and *S. aureus* is summarized in table 1. Based on the results, it can be concluded that the studied microbial species are common part of wastewaters. As it is seen, the density of microorganisms was higher in sludge compared to influent water and noticeably higher compared to effluent water.

We were also interested in the portion of resistant *E. coli* (Figure 1) and *S. aureus* (Figure 2) strains in wastewaters and sludge. In the case of *E. coli*, the resistance to 5 different antibiotics was tested in accordance to European (EUCAST) as well as to US (CLSI) resistance breakpoints. It is obvious that the strains were resistant to β-lactams, mainly to ampicillin. In the case of inflow water to WWTP B, there was detected 50% of coliforms resistant to ampicillin, 77% to gentamycin, 88% to ciprofloxacin and 51% to tetracycline. Wastewaters from hospitals and almshouse were a source of ampicillin, gentamycin and ciprofloxacin resistant coliforms; however their portion was higher than 50%.

Table 1 - Representation of total coliforms and staphylococci, *E. coli* and *S. aureus* (log CFU/ml; CFU/g) in wastewaters and sludge (CFU – colony formation units)

	A inflow	A sludge	A outflow	B inflow	B sludge	B hospital	B almshouse	C hospital
total coliforms	3.0	6.8	0.6	4.0	7.1	6.4	5.6	3.5
E. coli	2.4	6.1	0	3.2	6.6	4.5	0	0
total staphylococci	2.3	2.5	0	2.7	2.2	2.1	2.7	1.7
S. aureus	2.0	1.9	0	2.0	1.6	2.0	2.0	1.4

E. coli occurred only in water from hospital and in inflow water in WWTP B. From these strains, more than 70% were resistant to all 5 tested antibiotics. Ampicillin, gentamycin and ciprofloxacin were the less effective (there was more than 90% resistant *E. coli* strains). So, the hospital *E. coli* strains can be indicated as multi-resistant carriers. In inflow water no chloramphenicol resistant *E. coli* were detected. However, the prevalence of resistants to other antibiotics was higher than 50%.

In the case of wastewater from the WWTP A, lower prevalence of resistant *E. coli* strains to tested antibiotics was noticed. Resistance to chloramphenicol or tetracycline was not observed. Moreover, in the effluent water no resistants were detected at all.

Comparing the prevalence of resistant *E. coli* strains in wastewater and in sludge, it can be concluded that majority of the bacteria were attached into the sludge in both WWTPs. The prevalence of ciprofloxacin, chloramphenicol and tetracycline resistant *E. coli* was higher than 50%. The efficiency of ampicillin and gentamycin was even weaker, since there were detected more than 4, respectively 6 log CFU/g of resistant compared to 7 or 6.5 log CFU/g of all *E. coli* strains.

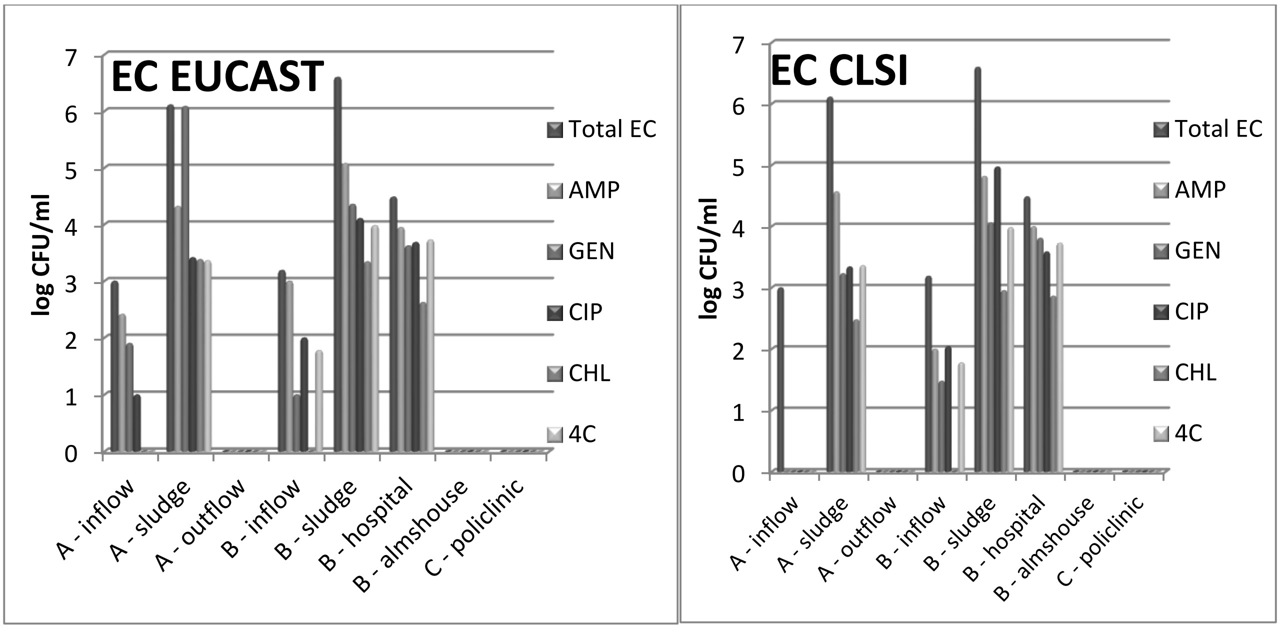

Fig. 1. Prevalence of resistant *Escherichia coli* (EC) in wastewaters and sludge according to EUCAST and CLSI resistance breakpoints.

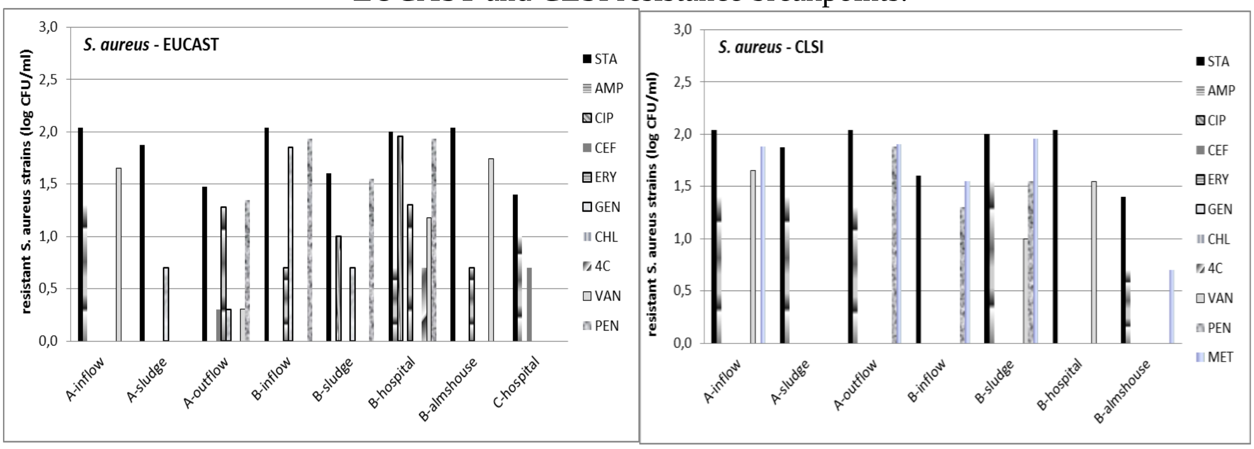

Fig. 2. Prevalence of resistant *S. aureus* in wastewaters and sludge according to EUCAST and CLSI breakpoints

In the case of *S. aureus*, there were no resistance to chloramphenicol and tetracycline in inflow and outflow water at all. On the other hand, high prevalence (91-95%) of penicillin resistant strains in two samples was detected and taking into account the US breakpoints, also high prevalence of methicillin-resistant strains (more than 90%).

In sludge from two different WWTPs, there were 37 or 44% resistant strains to gentamycin. Moreover, in WWTPs in city B, there were also resistant strains to ciprofloxacin (62%) and penicillin (96%). Contrary, there were no resistant strains to ampicillin, cefoxitin, erythromycin, chloramphenicol, tetracycline and vancomycin in any sludge. Taking into account the American breakpoints, the portion of penicillin-resistant strains decreased to 81%, however, there were detected 96% of methicillin-resistant strains in WWTPs in city B.

In wastewaters from hospitals, only gentamycin and chloramphenicol were totally efficient, taking into account the European limits. In the case of US limits, there were detected resistant *S. aureus* strains only to ampicillin (50-77%), vancomycin (50%), penicillin (77%) and methicillin (50-98%). It was noteworthy that the wastewater from the alms-house was not a significant source of resistant *S. aureus* strains. Only erythromycin and vancomycin resistant strains occurred in the case of European limits and in the case of American limits there were only 76% of vancomycin resistant strains.

4. CONCLUSIONS

Presence of antibiotic resistant or multi-resistant coliforms and staphylococci in wastewater represents a real threat for human health. Chloramphenicol and cefoxitin, respectively tetracycline was the most effective antibiotics; however the efficiency was not unconditional. Based on the results, it can be also concluded that majority of microorganisms flowing into WWTPs are partially attached to solid matrices into to the sludge and physically or chemically inhibited during separate treatment technological processes at WWTPs.

ACKNOWLEDGEMENT

This work was supported by the Slovak Research and Development Agency under the contract No. APVV-0122-12.

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УДК 628.543

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ДОСЛІДЖЕННЯ ЕЛЕКТРО - ТА БАРОМЕМБРАННОЇ ПЕРЕРОБКИ СТІЧНОЇ РІДИНИ ГІДРОРОЗРИВУ

При видобуванні нетрадиційних вуглеводнів на одну серію гідророзривів пласту (ГРП) в середньому витрачається 10000 м^3 води та утворюється 5000 м^3 сильно забрудненої стічної відпрацьованої рідини [1]. Ці рідкі відходи є головною екологічною проблемою регіонів видобування нетрадиційних вуглеводнів [2].

Метою дослідження є пошук шляхів створення маловідходної технології очистки відпрацьованої крекінгової рідини з можливістю подальшого використання отриманих продуктів.

Склад відпрацьованої фрекінгової рідини на свердловині «Біляївська-400» у Харківській області наведено у табл. 1.