

CTX-M-15 Type Extended-Spectrum Beta-Lactamase-Producing *Enterobacter cloacae* In Bioaerosol of a Municipal Solid Waste Recycling Plant: A New Possible Reservoir of Infection?

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Abstract

Background: Although there are many reports of antibiotic resistant microorganisms released from the treated municipal wastewater, less attention has been given to other waste treatment activities. We report the occurrence of antibiotic resistant strains isolated from the working setting of a solid waste recycling plant in Italy which serves 195 municipalities.

Methods: To assess microbial contamination levels, several sampling points were chosen for their relevance in evaluating exposure of the workers to biological risk, especially during indoor operations. Active sampling was carried out using the Surface Air System and Rodac plate method was applied to surface sampling. Species identification and antimicrobial susceptibilities were obtained by Vitek2 System (bioMérieux, France) and by the agar-diffusion method. The presence of resistance genes was investigated by multiplex PCR assay.

Results: In all sampling points a significant contamination of aerosol was detected, although the total microbial counts resulted within the limit proposed by the National authority (10000 CFU/sqm). The counts of *Enterobacteriaceae* were always above the limit of 100 CFU/sqm and strains of *Enterobacter cloacae* resistant to third generation cephalosporins were detected, carrying the *bla*CTX-M15 gene.

Conclusions: The contamination of workplace with multidrug-resistant strains is a crucial area for public health actions to avoid the conjunction of factors that promote evolution and spread of antibiotic resistance.

Keywords: CTX-M-15; *Enterobacter cloacae*; Bioaerosol; Solid waste

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Introduction

The emergence and spread of antimicrobial-resistant bacteria is a worldwide health concern, due to the intensive therapeutic use and misuse of antimicrobial agents in humans, as well as their therapeutic or sub-therapeutic use for prophylaxis and growth promotion in food animals [1].

Although there are many reports of antibiotic resistant microorganisms released from treated municipal wastewaters [2], as well as from livestock activities [3], less attention has been given to other waste treatment activities. Solid waste may be contaminated with used diapers from healthcare facilities as well as by expired medicines, thus promoting the interchange

of antibiotic resistant properties among pathogenic and environmental bacteria through horizontal gene transfer.

The biohazard related to waste treatment plants has been included by the European Agency for Safety and Health at Work among the 10 most important new and increasing biological risks [4]. All activities in solid waste management involve risk, from the point where workers handle wastes for collection or recycling, to the point of the processing of wastes, up to ultimate disposal. The major health problems observed in workers are caused by bioaerosols, causing upper airway inflammation and pulmonary disease, allergic reactions, irritation of the eyes and mucous membranes but also gastrointestinal infections [5]. Recently, the presence of tetracycline-resistant bacteria in nasal flora of

hog breeders, due to the inhalation of contaminated bioaerosol produced by hog manure has been reported [6], leading to the hypothesis of their possible spread to the general population. Moreover, airborne dispersal of bioaerosols from waste treatment activities could affect neighbouring facilities or residents, leading to health concerns.

Material and Methods

In this study, to assess the exposure to biological risk for workers in a municipal solid wastes recycling plant, serving as collection center for Tuscany and also areas of other regions in Italy, an environmental microbiological monitoring was carried out during the summer and winter seasons of the years 2011 and 2012.

Four sampling points were chosen for their relevance for the workers exposure, especially during indoor operations: the waste reception area, the automatic plastic selection, the manual glass selection and one point in the center of the plant. Active air sampling was carried out using the Surface Air System and Rodac plate method was applied to the surfaces sampling, according to the previously described protocol [7]. The samples were collected three times for each season during the years 2011 and 2012. Species identification and antimicrobial susceptibilities were obtained by Vitek2 System (bioMérieux, France) and by the agar-diffusion method; results were interpreted according to the EUCAST clinical breakpoints. In particular, for phenotypic detection of *AmpC* production, cefoxitin-EDTA disk diffusion test and cefoxitin-cloxacillin disk diffusion test were performed as described by Black et al. [8]. The presence of resistance genes were investigated by multiplex PCR assay with primers designed to amplify the following genes: *blaIMP*, *blaVIM*, *blaNDM*, *blaSPM*, *blaAIM*, *blaDIM*, *blaGIM*, *blaSIM*, *blaKPC*, *blaBIC*, *blaOXA-48*, *blaTEM*, *blaSHV* and *blaCTX-M* [9-11]. The sequencing of PCR product on both strands was performed for the identification of the gene variants.

Results

In all sampling points of the waste recycling plant the counts of Enterobacteriaceae were always above the limit of 100 CFU/m³. The species most frequently isolated were bacteria ubiquitous in the environment (for example: *Serratia* spp., *Klebsiella oxytoca* and *Citrobacter freundii*), but potential human pathogens strains, such as *Klebsiella pneumoniae* and *Enterobacter cloacae*, were detected showing in some instance multiple antibiotic resistance (**Table 1**).

In particular, resistance to third generation cephalosporins were observed by the agar-diffusion method in *E. cloacae* strains isolated in the aerosol collected in the center of the confinement building of the facility. Moreover, some strains showed reduced susceptibility to fourth-generation cephalosporins (cefepime) probably due to the presence of Extended-Spectrum Beta-Lactamase (ESBL) production. In 10 (50 CFU/m³) on 25 (75 CFU/m³) analysed strains, the multiplex PCR revealed the presence of the CTX-M gene and the sequencing of the PCR product identified the CTX-M-15 gene variant. No other resistance genes were identified.

The CTX-M-15 gene was found in *E. cloacae* strains isolated from the recycling process, and the strains were resistant to cefotaxime (MIC, 32 mg/L), to ceftazidime and aztreonam (MIC, 4 mg/L, MIC, 8 mg/L respectively), tobramycin (MIC, 4 mg/L), and intermediate to cefepime (MIC, 3 mg/L), but susceptible to imipenem (MIC, 0.25 mg/L). Clavulanate restored the activities of cefotaxime, ceftazidime, and cefepime in all CTX-M-15 producing strains.

Discussion

Several members of the Enterobacter genus, are naturally resistant to amoxicillin and cephalosporins, for example, *Enterobacter cloacae* produces a chromosomally encoded β -lactamase, also called cephalosporinase by the *bla_{AmpC}* gene expression. In general, the *bla_{AmpC}* expression is low but inducible in response to β -lactam exposure, that activates the *bla_{AmpR}* gene (transcriptional activator). During therapy, mutation in *bla_{AmpD}* may also lead to AmpC hyperinducibility (overexpression) or constitutive hyperproduction. Nevertheless, ESBL phenotype in *E. cloacae* may be mediated by *bla_{TEM}*, *bla_{SHV}*, *bla_{CTX-M}* and *bla_{OXA}* plasmid-encoded genes that confer resistance to oxymino-cephalosporins (cefotaxime, cefpodoxime, ceftazidime, ceftriaxone, and for some enzymes cefepime) and monobactam (aztreonam).

The prevalence of Enterobacteriaceae isolates resistant to third-generation cephalosporins in acute care hospitals is very high in Italy (about 40-50%), and the percentage of resistant isolates continues to increase in the other European countries [12]. Nevertheless the community is also strongly affected as recently reported by Andremont [13]. The community ESBL-producing enterobacteria fecal carriage rates has increased significantly everywhere and the CTX-M enzymes, especially CTX-M-15, are the dominant type with an estimated 35 million fecal carriers in Europe. Moreover, CTX-M-producing enterobacteria were newly reported in farm environment and in cultivated soils fertilized with manure [14] and in Switzerland enterobacteria identified as CTX-M-15 producers were isolated from rivers and lakes, leading to the hypothesis that synanthropic as well as water-associated birds should be considered a potential reservoirs and may be involved in transmission to humans [15].

In our study, the environmental microbiological monitoring carried out in a municipal solid wastes recycling plant found a significant contamination of aerosol by enterobacteria. Although the total microbial counts resulted within the limit proposed for these settings by the Italian Workers Compensation Authority (10000 CFU/m³) [16], the counts of Enterobacteriaceae were always above the limit of 100 CFU/m³. Of interest, *E. cloacae* strains, isolated in the aerosol collected in the center of the confinement building of the facility, resulted resistant to third generation cephalosporins and intermediate resistant to fourth-generation cephalosporins (cefepime). In *E. cloacae* strains, this phenotype may correspond to the simultaneous expression of a derepressed chromosomal *bla_{AmpC}* gene and to the Extended-Spectrum Beta-Lactamase (ESBL) production. The ESBL phenotype may be mediated by several plasmid-encoded genes such as *bla_{TEM}*, *bla_{SHV}*, *bla_{OXA}* and *bla_{CTX-M}*. The molecular methods applied to the study of resistant genes in *E. cloacae* strains isolated from the solid wastes recycling plant demonstrated the only presence

Table 1 Mean bacteria counts of isolates with antimicrobial resistance phenotype within the Enterobacteria.

Sampling site	Total coliform (CFU/m ³)	Prevalence of species isolated	AMI	GEN	TOB	CIRP	LEVOF	SAM	AMC	PTZ	FEP	CF	CTR	CTX	CPD	CAZ	IMI	MRP	AZT	RIF	PL	FO
Automatic plastic Selection	460	<i>Y. enterocolitica</i> (43%)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	200	0	0
		<i>E. cloacae</i> (33%)	0	0	0	0	0	0	150	0	0	150	0	0	150	0	0	0	0	150	0	150
		<i>K. pneumoniae</i> (24%)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	110	0	0
Manual glass selection	1180	<i>Serratia liquefacies</i> (41%)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	480	0
		<i>Klebsiella oxytoca</i> (31%)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	370	0	0
Plant center	800	<i>E. cloacae</i> (9%)	0	0	75	0	0	0	75	0	50	75	75	75	75	75	0	0	75	75	0	75
		<i>Serratia marcescens</i> (50%)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	400	0
		<i>Citrobacter freundii</i> (41%)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Waste reception area	140	<i>K. pneumoniae</i> (11%)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	15	0	0
		<i>Serratia liquefacies</i> (89%)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	75	0

Amikacin (AMI) Gentamicin (GEN) Tobramycin (TOB) Ciprofloxacin (CIRP) Levofloxacin (LEVOF) Ampicillin-sulbactam (SAM) Amox+clav (AMC) Piper-Tazobactam (PTZ) Cefepime (FEP) Cefoxitin (CF) Ceftriaxone (CTR) Cefotaxime (CTX) Cefpodoxime (CPD) Ceftazidime (CAZ) Imipeneme (IMI) Meropeneme (MRP) Aztreonam (AZT) Rifampicin (RIF) Polimixin (PL)

of the CTX-M-15 gene variant, the most prevalent gene among fecal carriers in Europe.

Conclusion

Although the data obtained in this study are only preliminary, the detection of CTX-M-producing enterobacteria in the inanimate environment of a solid waste plant may represent a risk of colonization or infection for the exposed workers, as well as of the environmental spread, through bioaerosol, of multi-drug resistant bacteria.

Contamination of the workplace with multidrug-resistant strains is of public health concern and may require action to ensure highest level of safety and health at work. Screening for genes associated with ESBL production may have an important impact on monitoring the resistant strains which have endemic potential and this could be the goal of our future studies, introducing the

screening into health surveillance of workers of this plant.

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Competing Interests

All authors have no conflict of interest to declare.

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