Original article

Class 1 integrons in drug-resistant *E. coli* and *K. pneumoniae* from blood stream infections

Integronii de clasă 1 la tulpinile de *E. coli* şi *K. pneumoniae* izolate din hemoculturi şi rezistente la antibiotice

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Abstract

Objective. The aims of our study were to assess the presence of class 1 integrons in drug-resistant E. coli and K. pneumoniae from blood stream infections (BSI) and to analyze their association with epidemiological and clinical characteristics. Methods We retrospectively studied BSI, classified as community-acquired (CA), health care associated (HCA), and hospital-acquired (HA), caused by antibiotic-resistant E. coli and K. pneumoniae strains from patients admitted to three hospitals in Bucharest between January 2009 and May 2011. Patient characteristics were also collected and analyzed. The susceptibility testing was performed with disk-diffusion and automated methods. Integrons were detected by PCR targeting the integrase gene. Results. Three hundred and twenty-five Gram negative BSI isolates were identified. Of these, the most common species were 163 E. coli (50%), and 67 K. pneumoniae (20%). Class I integrons were identified in 42 of 77 drug-resistant E. coli and 18 of 26 K. pneumoniae drug-resistant isolates. The presence of integrons in E. coli and K. pneumoniae BSI isolates was significantly associated with trimethoprim-sulfamethoxazole resistance ($p<0.01$ respectively 0.01). Integron-positive isolates were also more likely to be multi-resistant. The int1 gene was detected among 17 (50%) CA, 17 (59%) HCA and 24 (67%) HA infections. Conclusion. The proportions of drug resistance in class 1 integron-positive strains were higher than in those not carrying integrons, for both E. coli and K. pneumoniae. Integrons were significantly associated with trimethoprim-sulfamethoxazole resistance. Most of resistant E. coli and K. pneumoniae isolated either in CA, HCA or HA bacteremias are carrying class 1 integrons, which are easily transferable.

***Keywords**: class 1 integrons, bacteremia, E. coli, K. pneumoniae*

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Rezumat

Obiectivele studiului au fost evaluarea prezenței integronilor de clasa 1 la tulpini de E. coli şi K. pneumoniae rezistente la antibiotice izolate din bacteriemii şi analizarea asocierii prezenței acestora cu caracteristici epidemiologice şi clinice. Metode Am studiat retrospectiv bacteriemiile clasificate în comunitare, asociate îngrijirilor de sănatate şi nozocomiale, produse de E. coli şi K. pneumoniae, la pacienți internați în trei spitale din Bucureşti între ianuarie 2009 şi mai 2011, pentru care au fost inregistrate si analizate datele clinice. Testarea susceptibilității la antibiotice a fost realizată prin metoda difuzimetrică şi metode automatizate. Integronii de clasa 1 au fost detectați prin PCR țintind gena integrazei. Rezultate Au fost identificați 325 bacili Gram negativi izolați din hemoculturi în perioada studiată. Dintre aceştia cei mai frecvenți au fost E. coli, 160 (50%) şi K. pneumoniae, 67 (20%). Integronii de clasă 1 au fost identificați în 42 din 77 tulpini de E. coli rezistente la anti-microbiene şi 18 din 26 tulpini de K. pneumoniae rezistente la anti-microbiene. Prezența integronilor la tulpinile de E. coli şi K. pneumoniae izolate din hemoculturi a fost semnificativ statistic asociată cu rezistența la trimetoprim-sulfametoxazol ($p<0.01$ respectiv $p=0.01$). Tulpinile la care a fost detectată prezența integronilor au fost mai frecvent asociate cu multirezistența. Gena integrazei a fost evidențiată la 17 (50%) din tulpinile de E. coli si K. pneumoniae izolate din bacteriemii comunitare, 17 (59%) din infecții sistemice asociate îngrijirilor de sănătate şi 24 (67%) din bacteriemiile dobândite în spital. Concluzii Proporția rezistenței la anti-microbiene la tulpinile cu integroni de clasă 1 a fost mai mare decât în rândul tulpinilor fără integroni, atît pentru E. coli cât şi pentru K. pneumoniae. Prezența integronilor în tulpinile de E. coli şi K. pneumoniae izolate din hemoculturi este semnificativ asociată cu rezistența la trimetoprim-sulfametoxazol Cele mai multe tulpini de E. coli şi K. pneumoniae, rezistente izolate din bacteriemii comunitare, asociate îngrijirilor de sănătate şi nozocomiale poartă integroni de clasă 1, care sunt transferabili cu uşurință.

***Cuvinte cheie**: integroni de clasa 1, bacteriemie, E. coli, K. pneumoniae*

Introduction

Blood stream infections (BSI) are an important cause of morbidity and mortality in hospitalized patients, who acquire their infection in the community or hospital settings. Gram-negative bacilli (GNB) are the major cause for the community-acquired (CA) bacteremias and a major cause of death in hospital acquired (HA) BSI.(1) In a recently published study from Spain, GNB were more frequent in health care associated (HCA) bacteremias than in CA and HA infections (2).

Resistance of GNB to antimicrobial agents is caused by many different genetic determinants. Initially, multidrug resistance was not anticipated, because the appearance of multiple mutations responsible for antimicrobial resistance was considered unlikely. It is now clear that bacteria were ready for such a challenge and had already developed the genetic tools to confer multidrug resistance, including integrons (3)**.** The horizontal transfer of drug resistance genes involves systems such as plasmids, transposons and site-specific recombination systems named integrons (4)**.** Integrons were described in the late 1980s (5) and they are major vectors of antimicrobial multiresistance in GNB (6), because although not mobile themselves, the integrons can be carried by transposons and plasmids (7, 8)**.**

At present, integrons can be classified into mobile integrons (MI) and chromosomal integrons (CI), described in the late 1990s (3, 9)**.** There are five classes of MI, but the most clinically important and widespread is class 1. They are detected mainly in GNB, although class 1 integrons might also be found in Gram positive organisms (3, 9)**.** The sedentary CI have been discovered in the late 1990s and they are not involved in the resistance phenotype, but seem to be part of an adaptive genetic system, maintained long time in the genome of *Gram-negative* bacteria, helping bacteria to adapt to the changing world. On the other hand, these chromosomal elements are the source of the MI and of their antibiotic resistance genes (3)**.** In the present study we aimed to identify the presence of class 1 integrons

in *E. coli* and *K. pneumoniae* isolated from BSI and resistant to at least one antimicrobial drug and to look for their association with certain epidemiological and clinical characteristics.

Material and method

Strain and clinical data collection

We reviewed 325 BSI caused by drug-resistant GNB strains from patients admitted to two tertiary infectious diseases facilities and in one general hospital in Bucharest, Romania between January 2009-May 2011. Seventy-seven *E. coli* isolates and 26 *K. pneumoniae* isolates were included in our study, and epidemiological and clinical data were collected when the clinical records were available.

The data collected included: setting of infection; demographic characteristics; underlying diseases; source of BSI; prior antimicrobial use; major surgery and history of urinary tract infections (UTI) in the preceding 3 months of the BSI episode. The BSI episode was defined as HA if the first positive blood culture occurred >48h after admission; all other episodes were considered community-onset and subsequently classified as HCA, if the patient had been hospitalized within the preceding 90 days or was undergoing hemodialysis within the previous 30 days. The remaining episodes were classified as CA. The study was approved by the ethics committee of the three hospitals.

Antimicrobial susceptibility testing

Bacteria were identified by classic techniques or automated methods: miniAPI, VITEK2C. The susceptibility testing was performed with disk-diffusion, automated methods (CMI-ATB Expression/BioMerieux, VITEK2C), according to (CLSI) guidelines (10, 11)**.** The clavulanic acid synergy test was used to identify ESBL production. Antimicrobial agents tested, included aminoglycosides, beta-lactams +/- beta-lactamase inhibitors, carbapenems, cephems, monobactams, quinolones, cyclines, trimethoprim-sulfamethoxazol and colistin in isolates resistant to multiple antimicrobial drugs. Multiple drug resistance (MDR) was defined as resistance to one or more agents in three or more classes of tested drugs. Strains with intermediate susceptibility were classified as resistant.

DNA isolation and PCR amplification for integron

DNA was isolated by the QIAamp DNA Blood Mini Kit, following manufacturer's instructions. Class 1 integrons were detected by PCR with specific primers using the protocol reported by Ajiboye. (12) Primer sequences were used as previously published: CCTCCCGCACGATGATC for IntI1F and TCCACGCATCGTCAGGC for IntI1R. PCR was performed in 25 µl volumes containing 2 µl of template DNA, 1.5 mM $MgCl_2$, 0.4 mM (each) deoxynucleoside triphosphates (dNTPs), 1 U of Invitrogen *Taq* polymerase, 1x Invitrogen PCR buffer and 2 µM of each primer. PCR amplification was performed with GeneAmp PCR System 9700 thermal cycler (Applied Biosystems). PCR conditions for class 1 integrons consisted of an initial denaturation step of 5 min at 95°C followed by 35 cycles of denaturation for 1min at 94°C, annealing for 1min at 55°C, extension for 2 min at 72°C and a final extension step for 7 min at 72 °C. PCR products were resolved by electrophoresis at 100 V for 1 h on 2% agarose gel with 1x TAE buffer containing ethidium bromide and were visualized under UV light.

Statistical analysis

In order to identify the characteristics associated with the presence of class 1 integrons in our study population, we tested for differences between isolates with and without class 1 integrons using the chi-square test for categorical variables and the Mann-Whitney U test for continuous variables.

Results

Between January 2009 and May 2011, 325 BSI isolates were identified. The most common isolates were *E. coli* (163, or 50%), and *K. pneumoniae* (67, or 20%). One hundred and nineteen (73%) *E. coli* and 39 (58%) *K. pneumoniae* were resistant to at least one antimicrobial class. Here, we examined 103 resistant isolates (77 *E coli* and 26 *K. pneumoniae*).

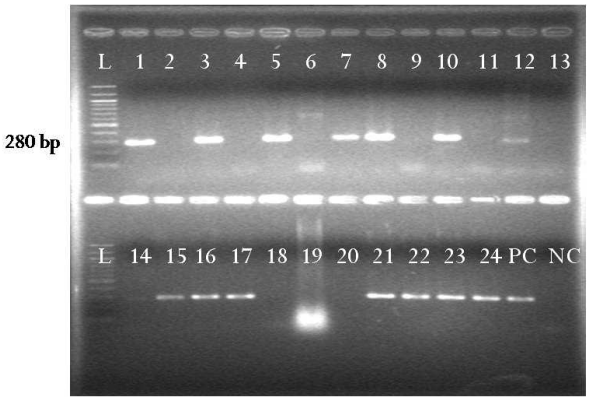

Figure 1. Agarose gel electrophoresis of integrase gene PCR amplification products: Lane L – 100 base-pair DNA ladder; lanes 1-24 drug resistant isolates; PC – positive control; NC – negative control. Isolates from lanes 1, 3, 5, 7, 8, 10, 15-17, 21-24 had the class 1 integrase gene.

The PCR amplification products were visualized by agarose gel electrophoresis (*Figure 1*). IntI1 gene was detected in 42 (54%) *E. coli* and in 18 (69%) *K. pneumoniae.*

The proportions of drug resistance in class 1 integron-positive strains were higher than those that did not carry the integrons, for both *E coli* and *K. pneumonia* (*Table 1*). However, quinolone resistance was more frequent in class 1 integron-negative *E. coli* isolates (57% vs 43%). Twenty-nine (76%) trimethoprim-sulfamethoxazole-resistant *E. coli* strains were carrying intI1 gene compared to 9 (24%) in those susceptible to trimethoprim-sulfamethoxazole ($p<0.01$). For *K pneumoniae* trimethoprim-sulfamethoxazole-resistant strains, the gene encoding class 1 integrons was detected in 16 (84%) strains, compared to 3 (16%) in those susceptible to trimethoprim-sulfamethoxazole ($p=0.01$).

Multidrug resistance phenotypes were found in 37 (48%) of *E coli* isolates and 22 (85%) of *K. pneumoniae* isolates. The genes encoding class 1 integrons were found in *E. coli* and *K. pneumoniae* in 28 (76%) MDR and 9 (24%) non-MDR ($p<0.05$) respectively 18 (82%) MDR and 4 (18%) non-MDR ($p<0.05$).

Class 1 integrase genes were detected in 17(63%) ESBL-producing *E. coli* strains and 10 (37%) ESBL-negative *E. coli* strains ($p>0.05$). ESBL-producing *K. pneumoniae* strains were found to carry class 1 integrase gene in 17 (74%) isolates, compared with 6 (26%) ESBL-negative strains ($p>0.05$).

We could collect clinical and epidemiological data for 99 patients, from 34 CA, 29 HCA and 36 HA infections. The *int1* gene was detected among 17 (50%) CA, 17 (59%) HCA and 24 (67%) HA infections ($p>0.05$). Although it did not reach statistical significance, *K. pneumoniae* strains carrying class 1 integrase gene were identified mainly from HCA and HA infections, while in *E. coli* class 1 integrase gene was identified in similar proportion in CA, HCA and HA infections *(Table 2).*

Patient characteristics, clinical features and in-hospital mortality of BSI caused by resistant *Enterobacteriaceae* are shown in *Table 3*. Strains carrying class 1 integrons were detected in patients with median age of 67 years (range 4-90 years), compared with 63 years (range 4-87 years) in patients infected with integron-negative. The most common source of BSI in this study was the urinary tract. In our study 44 (57%) *E. coli* strains and 7 (27%) *K. pneumoniae* were responsible for UTI. Class 1 integrons were present in almost half of these strains. There was no significant difference between the presence or absence of class 1 integrons among BSI GNB isolates from patients with recent surgery, antimicrobial use or recent UTI. Co-morbidities such as diabetes, chronic liver or kidney disease, cancer, HIV infection or alcohol abuse were seen in similar proportions in the two groups.

Discussion

The class1 integrons are found in clinical isolates and most of the known antimicrobial-resistance-gene cassettes belong to this class. More than 130 different cassettes have been

Table 1. Antimicrobial resistance for 103 resistant *E.coli* and *K.pneumoniae* isolated from BSI

	***E coli* N=77 Class 1 integron**			***K pneumoniae* N=26 Class 1 integron**			**Total Class 1 integron**		
Resistance N (%)	**Positive N (%)**	**Negative N (%)**	**p value**	**Positive N (%)**	**Negative N (%)**	**p value**	**Positive N (%)**	**Negative N (%)**	**OR (CI 95%) p**
Penicillins N=96 (93)	40 (57)	30 (43)	>0.05	18 (69)	8 (31)	NA	58 (60)	38 (40)	3.82 (0.61-30.1) p = 0.1
Penicillins/beta-lactamase inhibitors AMC/TCC/TZP N=66 (64)	26 (62)	16 (38)	>0.05	17 (71)	7 (29)	>0.05	43 (65)	23 (35)	2.20 (0.90-5.44) p = 0.09
Third generation cephalosporins CAZ/CRO/CTX N=50 (49)	17 (63)	10 (37)	>0.05	17 (74)	6 (26)	>0.05	34 (68)	16 (32)	2.21 (0.92-5.34) p = 0.08
Forth generation cephalosporins FEP N=38 (56)	14 (70)	6 (30)	>0.05	15 (83)	3 (17)	>0.05	29 (76)	9 (24)	OR* p = 0.03
Carbapenems IMP/MER/ETP N=4 (4)	2 (100)	0	>0.05	2 (100)	0	>0.05	4 (100)	0	OR* p = 0.1
Aminoglycosides GEN/TOB/AMK/NET N=49 (48)	17 (63)	10 (37)	>0.05	17 (77)	5 (23)	>0.05	34 (69)	15 (31)	2.44 (1.01- 5.96) p = 0.04
Quinolones CIP/LVX/NOR N=51 (50)	18 (43)	11 (57)	>0.05	16 (73)	6 (27)	>0.05	34 (67)	17 (33)	2.0 (0.84-4.81) p = 0.1
Trimethoprim-Sulfamethoxazol N=57 (61)	29 (76)	9 (24)	<0.05	16 (84)	3 (16)	<0.05	45 (79)	12 (21)	9.75 (3.6-29.21) p=0.001
One antimicrobial class	4 (25)	12 (75)	<0.05	0	1 (100)	>0.05	4 (23)	13 (77)	0.16 (0.04-0.61) p=0.03
Two antimicrobial classes	10 (42)	14 (58)	>0.05	0	3 (100)	<0.05	10 (37)	17 (73)	0.31 (0.11-0.83) p=0.01
Three antimicrobial classes	28 (76)	9 (24)	<0.05	18 (82)	4 (18)	<0.05	46 (78)	13 (22)	7.8 (2.88-20.41) p=0.001
ESBL-positive	17 (63)	10 (37)	>0.05	17 (74)	6 (26)	>0.05	34 (68)	16 (32)	2.21 (0.92-5.34) p=0.08
Total	42 (54)	35 (46)		18 (69)	8 (31)		60 (58)	43 (42)	

OR (odds ratio)* =OR undefined

Resistance was considered when the strain was resistant to at least one of:
- ampicillin/clavulanate (AMC), ticarcillin/clavulanate (TCC), piperacillin/tazobactam (TZP) for Penicillins/betalactamase inhibitors
- ceftriaxon (CRO), cefotaxim (CTX), ceftazidim (CAZ), for Third generation cephalosporins
- cefepime (FEP) for Fourth generation cephalosporins
- imipenem (IMP), meropenem (MER) or ertapenem (ETP) for Carbapenems

Table 2. The presence of Class 1 integrons according to the setting of infection

Class 1 integron positive isolates N (%)	Community acquired N=34	Health care associated N=29	Hospital associated N=36	p-value
E coli N=41	16 (39)	12 (29)	13 (32)	0.50
Klebsiella pneumoniae N=17	1 (6)	5 (29)	11 (65)	0.80
Total N=58	17 (29)	17 (29)	24 (42)	0.36

Table 3. Patient characteristics and clinical features of patients with BSI due to *E. coli* and *K. pneumoniae* carrying Class 1 integrons

	Class 1 integron-positive isolates N (%)	**Class 1 integron-negative isolates N (%)**
Male gender	38 (63)	21 (49)
Urinary source of bacteremia	29 (48)	22 (51)
Chronic renal disease	3 (5)	7 (16)
Chronic liver disease	8 (13)	8 (18)
Solid cancer	11 (18)	3 (7)
Hematological cancer	5 (8)	4 (9)
HIV infection	3 (5)	3 (7)
Alcohol abuse	5 (8)	2 (5)
Diabetes	14 (23)	13 (30)
Recent surgery (the preceding 3 months)	20 (33)	10 (23)
Recent antimicrobial use	22 (37)	16 (37)
Recent history of UTI	17 (28)	8 (19)
In hospital mortality	15 (25)	5 (12)
Total	60 (58)	43 (42)

identified and they carry antibiotic resistance genes responsible for resistance to most classes of antimicrobials, including beta-lactams, aminoglycosides, trimethoprim-sulfamethoxazole, quinolones, but also antiseptics like quaternary ammonium-compounds (13).

The occurrence of *intI1* gene among BSI drug-resistant *E. coli* and *K. pneumoniae* isolates in three hospitals in Bucharest was very high. More than half of drug-resistant *E. coli* and *K. pneumoniae* harbored class 1 integrons. Furthermore, integron-positive isolates were more likely to be multi-resistant than integron-negative isolates.

Class 1 integrons have been detected in GNB isolated from many animals, demonstrating that commensal strains from food-producing animals may be an important source of integrons carrying antibiotic resistance genes (12, 14).

We found that a large proportion of trimethoprim-sulfamethoxazole-resistant *E. coli* (76%) and *K. pneumoniae* (79%) carried class 1 integrons. Integrons are frequently found among uropathogenic *E. coli* (15). Although we did not look for the gene cassettes in the class 1 integrons, other studies have reported the presence of the *dfr* (dyhydrofolate reductase) gene

in high proportions of trimethoprim-sulfamethoxazole-resistant uropathogenic *E. coli*, which may account for the rapid emergence of trimethoprim-sulfamethoxazole resistance in UTIs from many communities. Class 1 integrons were found to be an important genetic element of resistance to trimethoprim-sulfametoxazol, which may be a trigger for the expression of the integrase, through the stress response system (SOS system). (16). The high frequency of BSI drug-resistant *E. coli* carrying class 1 integrons in this study demonstrates the importance of community-acquired UTIs as a major source of more serious hospital infections.

The presence of integrons is not associated with certain risk factors for BSI, like advanced age or various co-morbidities. Among these co-morbidities recent surgery in the preceding three months, diabetes and cancer have been associated with bacteremias due to Gram-negative organisms, regardless of the presence of the class 1 integrons.

The main limitation of our study is the absence of data on gene cassette arrangements in strains carrying class 1 integrons. In addition, due to the retrospective nature of our study there were missing clinical and epidemiological data and from the strains resistant to at least one antimicrobial family only 65% of *E. coli* isolates and 67% of *K. pneumoniae* isolates have been studied.

Conclusion

Most resistant *E. coli* and *K. pneumoniae* isolated either from CA, HCA or HA bacteremias are carrying class 1 integrons. Class 1 integrons are of crucial importance for the occurrence and transmission of multidrug resistance, and they are widely distributed in *E. coli* and *K. pneumoniae* BSI isolates in Bucharest. Therefore, they might play a role in the spread of resistance. The presence of integrons in the most common *Enterobacteriaceae* isolated from BSI is significantly associated with trimethoprim-sulfamethoxazole resistance.

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