

Relationship of drug resistance to phylogenetic groups of *E. coli* isolates from wound infections

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Abstract

Background: Drug resistance is a major problem in *Escherichia coli* isolated from surgical wound infections. In this study, we evaluated relationship between phylogeneticity and drug resistance.

Methodology: A total of 29 multi-drug resistant (MDR) *E. coli* isolates of known drug resistance genes and integron profile were selected for the present study. Triplex PCR was conducted for phylogenetic classification of these isolates into four established phylogenetic groups: A, B1, B2 and D. Statistical analysis was done to determine the association of different drug resistance genes and integrons with the phylogenetic groups.

Results: Most of the isolates (44.8%) belonged to phylogenetic group A followed by group B2 and D (24.1% each) and group B1 (6.9%).

Conclusions: There is a definitive relationship between drug resistance and various phylogenetic groups of *E. coli* infecting wounds. A shift towards phylogenetic group A might be observed with an increasing drug resistance profile.

Keywords: Drug resistance, *E. coli*, wounds, phylogenetic groups

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Introduction

Escherichia coli are primarily regarded as a cause of food-borne diarrhoeal diseases, but the importance of extraintestinal infections caused by *E. coli* cannot be underestimated. Extraintestinal *E. coli* mainly cause urinary tract infections (UTIs), surgical wound infections, and neonatal meningitis. Treatment of these infections has become a serious challenge for clinicians due to rapid development of antimicrobial drug resistance among these pathogens. Extraintestinal *E. coli* isolates causing surgical wound infections account for a loss of \$94-252 million per annum in the United States alone [1] with a major share incurred on the treatment of resistant isolates. The losses in developing countries may be even more, but no comprehensive data is available to assess this.

Drug resistance in *E. coli* could predict its phylogenetic background and virulence profile in a complex fashion [2]. The majority of extraintestinal *E. coli* isolates belong to the most virulent phylogenetic group B2 and to a lesser extent group D, whereas commensal *E. coli* mostly belong to the less

virulent groups A or B1 [3]. However, in some human *E. coli* isolates from UTIs, a shift away from the most virulent group B2 towards the less virulent groups A, B1 and D was observed with increasing drug resistance [4].

Much work has been done on the phylogenetic grouping of *E. coli* involved in UTIs, with special reference to their drug resistance profile [2,5]. The data show evidence of a relationship between phylogenetic groups and drug resistance in *E. coli* from UTIs. However, no such data is available for *E. coli* infecting wounds. Thus, the current study was designed to investigate the relationship between drug resistance and phylogenetic grouping in locally isolated *E. coli* from surgical wound infections.

Materials and Methods

Sample collection and DNA extraction

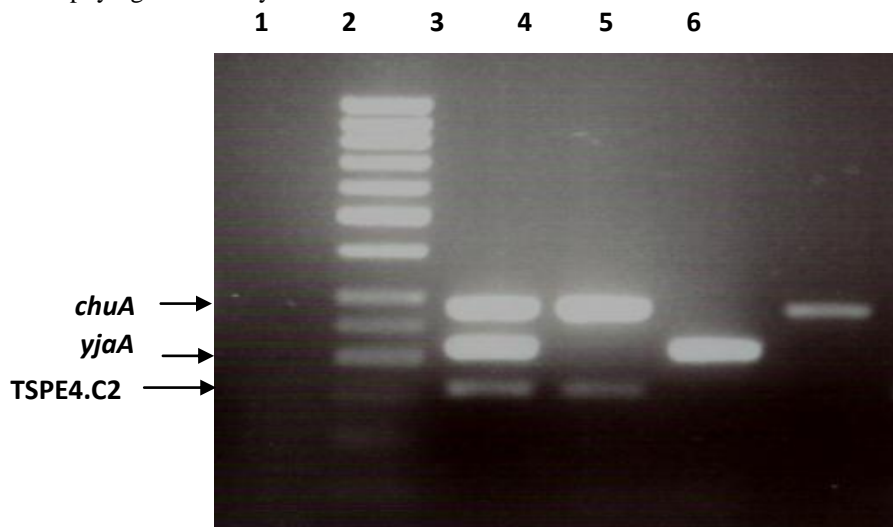
A total of 29 multi-drug resistant (MDR) *E. coli* isolates from surgical wound infections were selected from stock cultures for the present study. These isolates were of known drug resistance genes and integron profiles (Table 1). The culture stocks were

Table 1. Distribution of antimicrobial resistance genes among phylogenetic groups.

Antimicrobial groups	Gene targeted	B2 (n = 7)		D (n = 7)		A (n = 13)		B1 (n = 2)		Overall positive isolates
		Positive isolates	P value	Positive isolates	P value	Positive isolates	P value	Positive isolates**	P value*	
Ampicillin	<i>TEM</i>	5 (71.4%)	0.0012	5 (71.4%)	0.0012	10 (76.9%)	0.0001	1 (50%)	---	21 (72.4%)
	<i>bla_{TEM}</i>	2 (28.6%)	0.016	2 (28.6%)	0.016	1 (7.7%)	0.093	1 (50%)	---	6 (20.7%)
	<i>bla_{OXA}</i>	2 (28.6%)	0.016	1 (14.3%)	0.092	2 (15.4%)	0.016	0 (0%)	---	5 (17.2%)
Ciprofloxacin	<i>gyrA</i>	7 (100%)	0.0004	6 (85.7%)	0.0007	10 (76.9%)	0.0001	1 (50%)	---	24 (82.8%)
Chloramphenicol	<i>catP</i>	6 (85.7%)	0.0007	7 (100%)	0.0004	5 (38.5%)	0.0012	2 (100%)	---	20 (68.9%)
	<i>catA1</i>	7 (100%)	0.0004	6 (85.7%)	0.0007	6 (46.2%)	0.0007	1 (50%)	---	20 (68.9%)
Tetracycline	<i>tetA</i>	1 (14.3%)	0.092	0 (0.0%)	---	4 (30.8%)	0.0023	0 (0%)	---	5 (17.2%)
	<i>tetB</i>	5 (71.4%)	0.0012	5 (71.4%)	0.0012	7 (53.8%)	0.0004	1 (50%)	---	18 (62.1%)
Cephalosporins	<i>bla</i>	4 (57.1%)	0.0023	4 (57.1%)	0.0023	8 (61.4%)	0.0003	1 (50%)	---	17 (58.6%)
	<i>bla_{CTX-M-15}</i>	3 (42.9%)	0.0052	2 (28.6%)	0.016	3 (23.1%)	0.0052	0 (0%)	---	8 (27.6%)
Streptomycin	<i>aadA1</i>	0 (0.0%)	---	3 (42.9%)	0.0052	0 (0.0%)	---	1 (50%)	---	4 (13.8%)
Class 1 Integrons	<i>intI1</i>	2 (28.6%)	0.016	4 (57.1%)	0.0023	5 (38.5%)	0.0012	2 (100%)	---	13
Class 2 Integrons	<i>intI2</i>	3 (42.9%)	0.0052	3 (42.9%)	0.0052	1 (7.7%)	0.092	1 (50%)	---	8

*The p value could not be calculated due to the low number of isolates.

** Due to the low number of isolates, the percentage cannot be compared to that of other groups.

Figure 1. PCR based phylogenetic analysis of *E. coli* isolates.

Lane 1: Negative control without DNA.

Lane 2: Molecular weight marker (Fermentas SM0373; showing bands of 1031, 900, 800, 700, 600, 500, 400, 300, 250, 200, 150, 100 and 50bp).

Lane 3: *chuA* amplified product (279 bp); *yjaA* amplified product (211 bp); TSPE4.C2 amplified product (152 bp).

Lane 4: *chuA* amplified product (279 bp); TSPE4.C2 amplified product (152 bp).

Lane 5: *yjaA* amplified product (211 bp).

Lane 6: *chuA* amplified product (279 bp).

revived in tryptic soy broth (TSB, Merck, Germany) by 18 hours of incubation at 37°C. Total genomic DNA was extracted using the phenol-chloroform extraction method [6]. The integrity of the DNA samples was checked by electrophoresis on 1% agarose gel and purity was determined by ratio of A260/A280 spectrophotometrically. DNA samples were quantified with a fluorometer (DyNA QUANT 200, USA).

Multiplex PCR for phylogenetic grouping

A triplex polymerase chain reaction (PCR) was conducted to determine the phylogenetic grouping of isolates by targeting two genes, *chuA* and *yjaA*, and an anonymous DNA fragment, TSPE4.C2 [3]. Each 100 µl of PCR reaction mixture for multiplex PCR contained, in addition to 20 ng of DNA, 1.5 mM of MgCl₂, 70 µM of each dNTP, 1.0 µM of each primer and 10 U of *Taq* polymerase. Thermal cycler conditions were as follows: 94°C for 5 minutes followed by 30 cycles of 94°C for 30 seconds, 57°C for 30 seconds, and 72°C for 30 seconds. A final extension of 72°C for 7 minutes was performed at the end of each PCR.

Phylogenetic grouping

The phylogenetic tree described by Clermont and colleagues was used to classify all *E. coli* isolates into four phylogenetic groups: A, B1, B2 and D. The grouping decision was made based on the presence or absence of specified amplifications [3].

Results

All isolates showed different combinations of amplification products (Figure 1). Phylogenetic grouping revealed that group A was most prevalent with 13/29 (44.8%) isolates; seven (24.1%) isolates were found to belong to each of B2 and D; and B1 was least prevalent in these wound-infecting MDR *E. coli* as only two (6.9%) isolates belonged to this group.

The distribution of drug resistance genes and integrons in different phylogenetic groups is shown in Table 1. The most salient results were that all B2 group isolates carried mutated *gyrA* and *catA1* genes while all D group isolates were positive for *catP* gene. Among group A isolates, *TEM* and mutated *gyrA* genes were the most prevalent (76.9%). The comparison of intra-group percentages revealed that class 1 integrons were most prevalent (57.1%) in group D isolates, followed by group A (38.5%) and group B2 (28.6%). Contrary to this, class 2 integrons were equally distributed in groups B2 and D (42.9% each). Statistical analyses demonstrated that the distribution of class 1 and class 2 integrons among various phylogenetic groups (B2, D and A) had statistical significance ($p < 0.05$) with the exception of class 2 integrons in group A ($P > 0.05$).

Discussion

Millions of health and economic losses occur every year due to extraintestinal infections by *E. coli* [1]. Antimicrobial drug resistance is a major causative factor for these losses. *E. coli* antimicrobial drug resistance is strongly related to phylogenetic

grouping [2]. Extraintestinal *E. coli* isolates typically belong to phylogenetic groups B2 and D respectively, whereas commensal isolates belong to group A. The current study was designed to elucidate the effect of drug resistance on phylogenetic grouping in *E. coli* isolates from surgical wound origins. To our knowledge, no work has previously been done in this area.

We studied 29 MDR *E. coli* isolates of known drug resistance genes and integron profile. As extraintestinal *E. coli* (from surgical wound origins), our isolates were expected to belong mostly to group B2 [3]; however, this was not the case. The majority of our isolates (44.8%) belonged to the reportedly less virulent group A, followed by groups B2 and D (24.1% each), and group B1 (6.9%). This pattern is comparable to those noted in some previous studies of extraintestinal *E. coli* associated with UTIs in Russian females which reported that 55% of isolates belonged to group A, 23% belonged to groups B2 and D each, and no isolate represented group B1 [5]. In other studies, the resistant *E. coli* involved in UTIs showed a phylogenetic shift from group B2 toward group A [7,8]. The highest prevalence of group A in the present study demonstrated a similar phylogenetic shift in wound infecting *E. coli* isolates. We observed a general shift towards non-B2 phylogenetic groups. This phenomenon may be due to the presence of high drug resistance, as all of our *E. coli* were MDR isolates. Similar results have been reported in UTIs [8].

The relationship of drug resistance genes and integrons with all phylogenetic groups was statistically analyzed (Table 1). Statistical analyses demonstrated that the distribution of class 1 and class 2 integrons among various phylogenetic groups (B2, D and A) had statistical significance with the exception of class 2 integrons in group A. There are some reports available on *E. coli* from UTIs revealing that integrons are less frequent in B2 than in non-B2 isolates [7]. Our isolates showed the same pattern regarding class 1 integrons as only 28.6% of B2 isolates carried class 1 integrons in comparison to 57.1% and 38.5% isolates of groups D and A respectively. However, class 2 integrons were equally distributed among group B2 and D isolates (42.9% each) while only 7.7% isolates of group A carried class 2 integrons.

The relationship between phylogenetic groups and drug resistance genes has not previously been reported. We found that all B2 group isolates were positive for mutated *gyrA* and *catA1* genes while all

D group isolates were positive for the *catP* gene. Though most of the drug resistance genes were found in all phylogenetic groups (Table 1), there was no phylogenetic group found harboring a specific gene in a significantly higher proportion as compared with other groups.

We conclude that there is a definitive relationship between drug resistance and various phylogenetic groups of *E. coli* infecting wounds. A shift towards phylogenetic group A might be observed with an increasing drug resistance profile.

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