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Research Article

Molecular Profiling and Characterization of Integrons and Genotyping of *Escherichia coli* and *Klebsiella pneumoniae* Isolates Obtained from North Indian Tertiary Care Hospital

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Abstract

While the emergence of antibacterial drug resistance is a great emerging health issue that threatens the clinical usefulness of these drugs, it is important to study the spread of antibiotic resistance genes in order to understand the relationship between resistance gene pool and its mobilization through transposons and integrons. 125 cefoxitin-resistant *E. coli* (109) and *K. pneumoniae* (16) isolates were looked for the presence of integrons in order to illustrate the location of antibiotic resistance genes (especially bla_{ampC}). The genotyping was done by RAPD so as to find out genetic relatedness among isolates. 55.20% (69/125) isolates were found positive for integrons. 41 isolates showed single amplification band for CS region, 20 showed two bands, 4 showed three bands and four isolates showed multiple banding patterns. *Sul-1* was reported to be present in 3'CS, but we also observe 14/69 isolates that showed amplification for 5'CS-3'CS region but did not show presence of *Sul-1* (when detected by PCR). Out of 109 *E. coli* isolates, 91 could be typed by RAPD, while 18 were found untypable. Among 91 *E. coli* isolates, 33 were grouped in 15 clusters while the remaining 58 isolates showed unique banding patterns indicating genetic un-relatedness. Among 16 *K. pneumoniae* isolates, 14 were typed by RAPD and 2 isolates were found untypable. The higher rate of resistance to several classes of *β*-lactam antibiotics in integron-positive isolates is probably attributable to the association of *β*-lactamase genes with integron-carrying plasmids and hence suggests that antibiotic drug resistance is transmitting through these mobilizing agents. As evident from RAPD-typing, most patients in our hospital were infected with different clades of organisms, thereby demonstrating clonal diversity among isolates suggesting horizontal transmission of *bla* genes.

Introduction

Apart from well-known bacterial methods of mutations, the horizontal genetic transfer of genetic material within microbes plays a crucial role in the emergence of novel antibiotic resistance genes and their dissemination noticed worldwide. Rapid and widespread appearance of multidrug resistance patterns observed in closely as well as distantly related bacterial species are due to presence of mobile genetic elements, more specifically integrons.

Integrons are able to capture and exchange genes in a small mobile elements called cassettes by a process of site-specific recombination. They have been assigned to at least four different classes based on *int* integrase gene homology. Class 1 integrons are most prevalent in clinical isolates and usually associated with multidrug resistance phenotypes.

Horizontal transfer of genes is not limited only to resistance genes involving cell-to-cell transfer itself has become noteworthy after engagement of gene transfer from one DNA molecule to another by mobile genetic elements or transposable elements. They include insertion sequences, composite-, complex- and conjugative-transposons, transposing bacteriophages, integrons, and recently described ISCR (Insertion Sequence Common Region) elements [1].

Class 1 integrons usually consist of 5'-conserved segment (5'CS), 3'-CS and gene cassettes in between. The 5'CS region is conserved and composed of three key elements: integrase *int1* gene, primary recombination site *attI* and an outward oriented promoter Pc that directs transcription of captured gene cassettes. The 3'CS includes a *qacEΔ1* and a *Sul-1* gene; however this region can be

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Table 1: Antimicrobial Susceptibility of integron-positive and integron-negative *E. coli* and *Klebsiella pneumoniae* isolates.

Antimicrobial Agents	Integron-positive (n=69)	Integron-negative (n=56)
	% Resistance	% Resistance
β-Lactams		
Cefoperazone	91.43	76.36
Cefixime	91.43	87.27
Cefotaxime	95.71	92.73
Ceftazidime	78.57	83.64
Ceftriaxone	90.00	96.36
Cefepime	65.71	61.82
Cefpirome	78.57	72.73
Aztreonam	90.00	92.73
Imipenem	0	0
Fluoroquinolones		
Gatifloxacin	87.14	83.64
Ofloxacin	88.57	85.45
Aminoglycosides		
Gentamicin	61.43	49.09
Amikacin	47.14	29.09

variable in length and even be absent. The integron-captured gene cassettes share the specific structural characteristics and generally contain single gene and an imperfect inverted repeat at the 3' end of the gene called an *attC* site or 59-bp element.

It has been reported in the literature that integrons can be categorized as classic and non-classic integrons. Classic class 1 integrons contains an integrase gene (*int1*) in their 5'CS and *qacEΔ1+Sul-1* (encoding resistance to quaternary ammonium compounds and sulfonamides respectively) in their 3'CS region [2]. Moreover, non-classic class 1 integrons lack 3'CS region from the normal integron structure composition [3-5]. The *Sul-1* gene was found typically associated with class 1 integrons [2]. *Sul-2* gene was reported to be presenting adjacent to streptomycin resistance genes [6], whereas *Sul-3* gene has been recently illustrated to be linked to non-classic integrons [3,4,7].

While antibiotic resistance is a great emerging health issue, it is important to study the spread of antibiotic resistance genes to understand the relationship between resistance gene pool, its mobilization through transposons and integrons and dissemination among closely related as well as completely unrelated bacterial species.

Materials and Methods

Bacterial isolates

A total of 14, 129 samples were subjected to the department of microbiology, J. N. Medical College for routine culture and susceptibility testing during a period of 18 months and out of these, 1610 isolates were identified as *E. coli* and 455 as *K. pneumoniae* by the standard microbiological techniques [8]. A total of 109 *E. coli* & 16 *K. pneumoniae* (that were found resistant to cefoxitin) isolates were randomly selected and were previously characterized for bla_{ampC} genes [9].

Characterization of integrons

Cefoxitin-resistant *E. coli* and *K. pneumoniae* isolates that were previously characterized for the presence of bla_{ampC} genes [9] were now looked for the presence of integrons in order to illustrate the location of antibiotic resistance genes (especially bla_{ampC}). Presence of integrons was demonstrated by PCR amplification, using primer set 5CS-F1 (5'-ATG TTA CGC AGC AGG GC-3') and 3CS-R (5'-GGA ATT CGA CCT GAT AGT TTG GCT GTG-3') as forward and reverse primers respectively. PCR reaction mixture was prepared in a total volume of 25 µL comprising of 0.05 µl each of primers 5CS-F1 and 3CS-R, 12.5 µL master mixture, 9.9 µL DNase/RNase free distil water and 2.5 µL of template DNA. The reaction mixture so prepared was dispensed in PCR tube and placed in MJ-mini Bio-Rad thermal cycler (Bio-Rad, USA). PCR cycling conditions consist of an initial denaturation step at 95 °C for 3 min, followed by 30 cycles of 95 °C for 1 min, 58 °C for 1 min, 72 °C for 2 min, and a final elongation step at 72 °C for 10 min.

Genetic relatedness of cefoxitin-resistant isolates done by RAPD-PCR

RAPD-typing was done by using primer ERIC-2b (5'-AAG TAA GTG ACT GGG GTG AGC G-3') [10]. Reaction mixture was prepared by mixing 12.5 µL of master mix, 10.25 µL of distilled water, 0.25 µL of primer and 2.0 µL of template DNA. The PCR amplification was attained by placing reaction mixture containing tubes in MJ-mini Bio-Rad thermal cycler (Bio-Rad, USA). PCR cycling conditions consist of an initial denaturation step at 94 °C for 2 min, followed by 35 cycles of 94 °C for 1 min, 25 °C for 1 min, 72 °C for 4 min, and a final elongation step at 72 °C for 8 min. The results were analyzed by using Bio-Rad Gel documentation system (Bio-Rad, USA) and clustering was performed by Quantity One software provided by gel documentation system. Isolates of same species were typed in same batch and isolates of different wards were compared together to identify clonal spread.

Results

Antimicrobial susceptibility

All the integron-positive isolates were characterized by resistance to more than one antimicrobial agent tested as compared to integron-negative isolates. The range of the proportions of resistance for integron-positive isolates and integron-negative isolates for the third-generation cephalosporins (ceftriaxone, cefotaxime, ceftazidime) was 78.57%-95.71% and 83.64%-96.36% resistant, respectively. Moreover, all the isolates were found susceptible to imipenem. Details are shown in Table 1.

Characterization of integrons

41 isolates showed single amplification band when PCR was performed to amplify CS region. Out of these, three isolates showed amplification at 400 bp (lowest one), 11 isolates showed the amplicon at 800 bp, while three isolates showed the amplification of product at 900 bp. A major part of isolates, comprising of 24 isolates showed the amplification product at 1500 bp. It was the most common amplicon noted in our isolates. Details are shown in Table 2.

Out of three isolates that showed the amplicon at lowest position, two isolates were found negative for *Sul-1* gene. One isolate was found to carry bla_{SHV} only along with insertion sequence IS*26*. The

Table 2: Genetic organization of integron-positive isolates of *E. coli* and *Klebsiella pneumonia*.

Amplicon size Single band	Genes	Number of Isolates
400 bp	bla_{ampC}, bla_{CTX-M}, bla_{TEM}, bla_{SHV}, *Sul-1*, IS*26*	3
800 bp	bla_{ampC}, bla_{CTX-M}, bla_{TEM}, $bla_{SHV,}$ *Sul-1*, ORF*513*, IS*Ecp1*, IS*26*	11
900 bp	bla_{ampC}, bla_{CTX-M}, bla_{TEM}, $bla_{SHV,}$ *Sul-1*, ORF*513*, IS*Ecp1*, IS*26*	3
1500 bp	bla_{ampC}, bla_{CTX-M}, bla_{TEM}, $bla_{SHV,}$ *Sul-1*, ORF*513*, IS*Ecp1*, IS*26*	24
Two bands	bla_{ampC}, bla_{CTX-M}, bla_{TEM}, bla_{SHV}, *Sul-1*, ORF*513*, IS*Ecp1*, IS*26*	20
Three bands	bla_{ampC}, bla_{CTX-M}, bla_{TEM}, bla_{SHV}, *Sul-1*, ORF*513*, IS*Ecp1*, IS*26*	4
Multiple bands	bla_{ampC}, bla_{CTX-M}, bla_{TEM}, bla_{SHV}, *Sul-1*,ORF*513*, IS*Ecp1*, IS*26*	4

other two isolates harbor bla_{ampC}, bla_{CTX-M}, bla_{TEM}, and bla_{SHV}. As far as mobilizing elements are concerned (IS*26*, IS*Ecp1*, and ORF*513*), the *Sul-1*-harboring isolates also carried IS*26* while the other one do not bear any of the insertion sequence.

Among 11 isolates showing amplification for CS region at 800 bp, nine isolates were noticed to harbor *Sul-1* gene and two were found negative for the same. When analyzed for *bla* genes, three isolates were noted to carry combination of bla_{ampC}, bla_{CTX-M}, bla_{TEM}, bla_{SHV}, but these three isolates carry different array of mobile genetic element. *Sul-1* was noticed to be present in all three isolates, ORF*513* was observed in two, and IS*Ecp1* was found to be present in one isolate. IS*26* was also noticed in one isolate. The combination of bla_{ampC}, bla_{CTX-M}, and bla_{TEM} was observed in two isolates, where, IS*26*, IS*Ecp1*, *Sul-1*, and ORF*513* were present in one and the other three mobilizing elements except IS*Ecp1* were noticed in another isolate. Similarly, bla_{ampC}, bla_{CTX-M}, and bla_{SHV} combination was noticed in two isolates where ORF*513* was noticed to be absent in one isolate while rest of the three mobilizing elements were observed in both isolates. The combination of bla_{ampC} and bla_{CTX-M} was observed in two isolates and *Sul-1* was found absent in one of them. ORF*513* was present in both isolates, and the isolates that do not bear *Sul-1* showed the presence of IS*Ecp1* however, IS*26* was absent in both isolates. Single isolate that harbors only bla_{ampC} was found to carry all the four mobilizing elements. One isolate shows the amplification for CS region, but carries only ORF*513* and rest of the genes including *bla* genes and mobile genetic elements (screened in the present study) were found absent.

Three isolates showed CS amplification band at 900 bp, out of which *Sul-1* was noted only in one isolate. *Sul-1*-positive isolate harbors all the four *bla* genes studied i.e., bla_{ampC}, bla_{CTX-M}, bla_{TEM}, and bla_{SHV}. While, among mobilizing elements ORF*513* and IS*Ecp1* were present while IS*26* was found absent. In the remaining two isolates, one carries only bla_{ampC} along with ORF*513*, IS*Ecp1*, and IS*26*. The other *Sul-1*-negative isolate carries combination of bla_{ampC}, bla_{CTX-M}, and bla_{SHV} whilst other mobile genetic elements were absent.

The most common amplicon that was observed in amplification of Conserved Segment (CS) region was of 1500 bp, and was noticed in 24 isolates. *Sul-1* gene was observed in eighteen isolates and three isolates were noticed negative for it. Twelve isolates were found to carry combination of bla_{ampC} and bla_{CTX-M} genes and three out of them showed the absence of *Sul-1* gene. A total of five isolates showed the same array of *bla* genes and mobilizing element i.e., bla_{ampC}, bla_{CTX-M}, *Sul-1*, ORF*513*, and IS*Ecp1*. Two isolates showed the combination of bla_{ampC}, bla_{CTX-M}, *Sul-1*, IS*Ecp1*, and IS*26*. A single isolate was noticed that possess the combination of all four mobile genetic elements along with *bla* genes, i.e., the combination of bla_{ampC}, bla_{CTX-M}, *Sul-1*, ORF*513*, and IS*Ecp1*, and IS*26* was present. Out of nine bla_{ampC} and bla_{CTX-M} carrying-*Sul-1*-positive isolates, remaining one showed the presence of IS*Ecp1* as mobilizing agents. Among the three *Sul-1*-negative isolates, one isolate was found devoid of all mobilizing elements studied and carries only bla_{ampC} and bla_{CTX-M}. In the remaining two isolates, one showed only IS*26* as insertion sequence that can mobilize antibiotic resistance genes, while other one bears ORF*513* and IS*Ecp1*. The second most common combination of *bla* genes observed was that of bla_{ampC}, bla_{CTX-M}, and bla_{SHV}. All the four isolates demonstrating this combination of genes, showed similar pattern of mobilizing elements, i.e., the combination of *Sul-1* and IS*Ecp1* was noticed along with bla_{ampC}, bla_{CTX-M}, and bla_{SHV}. Combination of bla_{ampC}, bla_{CTX-M}, bla_{TEM} along with *Sul-1*, ORF*513*, and IS*Ecp1* was noticed in three isolates. The combination of all four *bla* genes was noticed in two isolates and they both displayed combination of mobilizing elements different from each other. One isolate showed combination of *Sul-1*, ORF*513*, and IS*Ecp1* while other one showed IS*26*, *Sul-1*, ORF*513* and IS*Ecp1* as mobile elements.

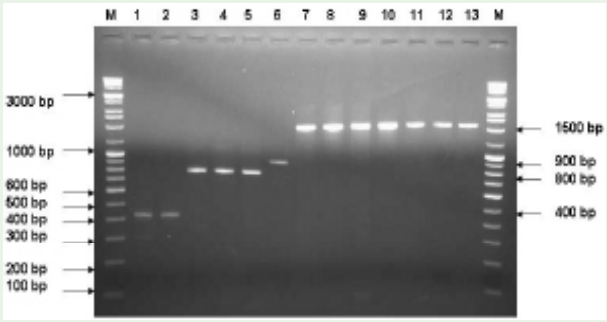

Figure 1(a): 2% agarose gel showing single amplicon observed in amplification of CS region.

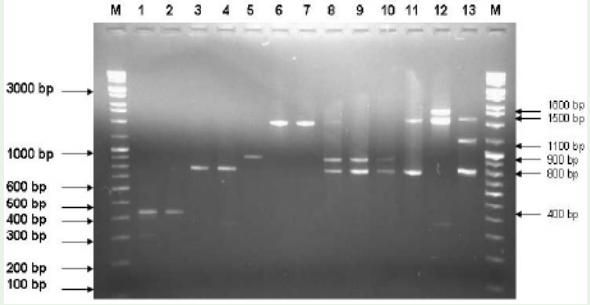

Figure 1(b): Agarose gel showing the amplification pattern of single, double and multiple amplicons noticed in amplification of CS region.

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Table 3: *bla* gene composition in various clusters of *E. coli* and *K. pneumoniae* isolates.

RAPD profile	No. of Isolates	Wards	*bla* genes
EC1	3	Surgery	bla_{ampC} ; bla_{ampC} + bla_{CTX-M} + bla_{TEM} ; bla_{ampC} + bla_{CTX-M}
EC2	2	Surgery	bla_{ampC} + bla_{SHV} ; bla_{ampC}
EC3	3	Surgery	bla_{ampC} ; bla_{ampC} + bla_{CTX-M} + bla_{SHV}
EC4	2	Surgery	bla_{ampC}+ bla_{CTX-M} + bla_{TEM} + bla_{SHV}
EC5	2	Surgery	bla_{ampC} + bla_{CTX-M} ; bla_{ampC} + bla_{CTX-M} + bla_{SHV}
EC6	2	Surgery	bla_{ampC} + bla_{CTX-M} + bla_{SHV} ; bla_{ampC}+ bla_{CTX-M} + bla_{TEM} + bla_{SHV}
EC7	2	Surgery	bla_{TEM} + bla_{SHV} ; bla_{ampC}+ bla_{CTX-M} + bla_{TEM} + bla_{SHV}
EC8	2	Surgery	bla_{ampC} + bla_{CTX-M} ; bla_{ampC}+ bla_{CTX-M} + bla_{TEM} + bla_{SHV}
EC9	2	Gynaecology	bla_{ampC} + bla_{CTX-M}
EC10	2	Gynaecology	bla_{ampC} + bla_{CTX-M} + bla_{TEM} ; bla_{ampC} + bla_{CTX-M} + bla_{SHV}
EC11	2	Gynaecology	bla_{ampC} + bla_{CTX-M} ; bla_{ampC}+ bla_{CTX-M} + bla_{TEM} + bla_{SHV}
EC12	2	Gynaecology	bla_{ampC} + bla_{CTX-M} ; bla_{ampC}+ bla_{CTX-M} + bla_{TEM} + bla_{SHV}
EC13	3	Medicine	bla_{ampC}+ bla_{CTX-M} + bla_{TEM} + bla_{SHV} ; bla_{ampC} + bla_{CTX-M} + bla_{TEM} ; bla_{ampC} + bla_{CTX-M} + bla_{SHV}
EC14	2	Medicine	bla_{ampC}+ bla_{CTX-M} + bla_{TEM} + bla_{SHV} ; bla_{ampC} + bla_{CTX-M} + bla_{SHV}
EC15	2	Medicine & Paediatrics	bla_{ampC}+ bla_{CTX-M} + bla_{TEM} + bla_{SHV}
KP1	2	Surgery	bla_{ampC} ; bla_{ampC}+ bla_{CTX-M} + bla_{TEM} + bla_{SHV}
KP2	2	Surgery	bla_{ampC} + bla_{TEM} + bla_{SHV} ; bla_{ampC}+ bla_{CTX-M} + bla_{TEM} + bla_{SHV}

Three isolates were found carrying the combination of bla_{ampC}, bla_{CTX-M}, bla_{TEM} and bla_{SHV}, however, combination of mobile genetic elements varied. Combination of *Sul-1* and IS*Ecp1* was observed in one isolate while in other ORF*513* replaces IS*Ecp1* (combination of *Sul-1* and ORF*513* was noted). Remaining isolate showed the combination of all four mobile elements i.e. IS*Ecp1*, *Sul-1*, ORF*513*, and IS*26* was noticed.

Sixty nine isolates showed amplification when PCR was performed for CS region and out of them twenty isolates showed two bands. Interestingly one isolate that do not harbor any of *bla* genes, showed two bands in amplification PCR performed for CS region and it carried *Sul-1* gene also thereby indicating the presence of Class 1 integron. Figure 1(a) and 1(b) shows the banding pattern observed in amplification of CS region.

Epidemiological typing of *E. coli* and *K. pneumoniae* isolates

Out of 109 *E. coli* isolates, 91 could be typed by RAPD, while 18 were found untypable. Among 91 *E. coli* isolates, 33 were grouped in 15 clusters (EC1 to EC15) while the remaining 58 isolates showed unique banding patterns indicating genetic unrelatedness. Maximum clustering was noticed in isolates obtained from surgery ward (EC1 to EC8). Surprisingly, no cluster was noticed in isolates obtained from orthopaedics ward. Figure 2(a-d) shows the RAPD patterns of *E. coli* isolates. *bla* gene composition of the isolates grouped in clusters was variable (Table 3), however, occurrence of bla_{ampC} was observed in all clusters except one isolate that harbours combination of bla_{TEM} & bla_{SHV}. Among 16 *K. pneumoniae* isolates, 14 were typed by RAPD and 2 isolates were found untypable. Figure 3 shows the RAPD profile

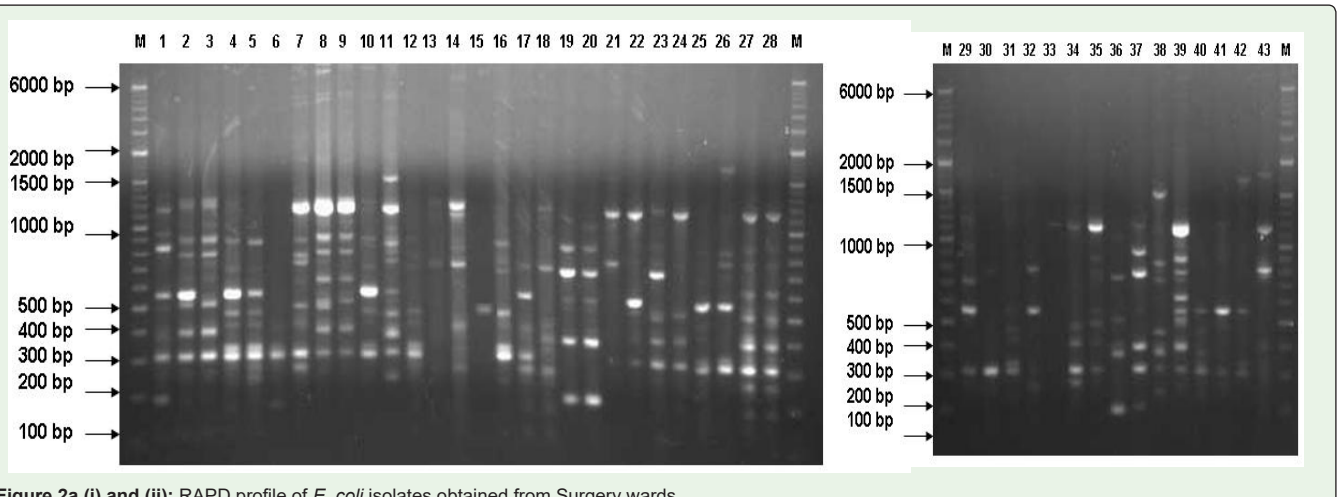

Figure 2a (i) and (ii): RAPD profile of *E. coli* isolates obtained from Surgery wards.

Citation: Sobia F, Shahid M, Jamali S, Khan HM and Niwazi S. Molecular Profiling and Characterization of Integrons and Genotyping of *Escherichia coli* and *Klebsiella pneumoniae* Isolates Obtained from North Indian Tertiary Care Hospital. SM Trop Med J. 2016; 1(1): 1003. https://dx.doi.org/10.36876/smtmj.1003

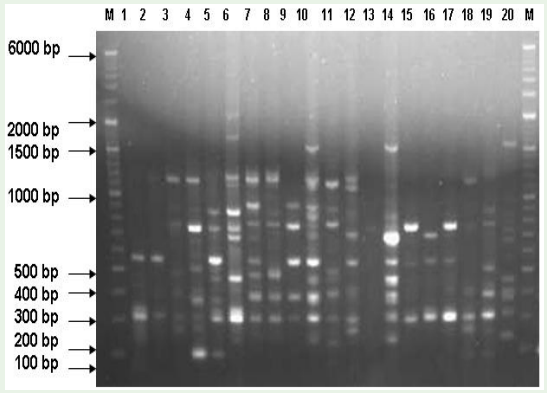

Figure 2b: RAPD profile of *E. coli* isolates obtained from Gynaecology wards.

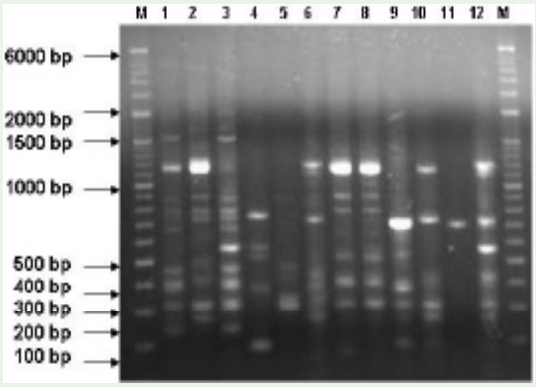

Figure 2d: RAPD profile of *E. coli* isolates obtained from Medicine and Paediatrics.

of *K. pneumoniae* isolates obtained from various wards. Out of 14 isolates, 4 were grouped in two clusters (KP1 & KP2) and 10 displayed unique banding patterns. Figure 4 shows the representative clusters of *E. coli* and *K. pneumoniae* isolates.

Discussion

The beneficial aspects of the introduction of antibiotics in the treatment of human infectious diseases is hampered by the emergence of bacterial resistance has become an ever-increasing problem that threatens the clinical usefulness of these drugs. Class 1 integrons has been identified as the primary source of antimicrobial resistance genes and is suspected to serve as reservoirs and exchange platform of resistance genes in a variety of Gram-negative bacteria. [11].

It has been reported that the antibiotic resistance was noticeably higher in class 1 integron-positive strains of P. aeruginosa as compared to those of class 1 integron-negative strains [12]. Recently [13] reported presence of integron in 53.9% Enterobacterial isolates. However, we have observed quite high percentage of β-lactam resistance in integron-negative isolates also, indicating that probably these genes are located on plasmids. More integron-harboring isolates were found resistant to fluoroquinolones than integron-negative isolates as previously reported by [14]. This may be due to the presence of integrons, which increase the mutation rate of the host cell [15], or the presence of genes on integrons that code for reduced membrane permeability or enhanced efflux [16].

The prevalence of antibiotic resistance is mainly due to the horizontal transfer of antibiotic-resistance genes, expressed by mobile genetic elements such as plasmids and transposons. One way for the spread of antibiotic resistance-encoding genes involves integrons. Class 1 integrons were significantly associated with resistance to all the antimicrobials tested. However, integron-positive strains were significantly more common among isolates showing resistance to β-lactam antibiotics. The higher rate of resistance to several classes of β-lactam antibiotics in integron-positive isolates is probably attributable to the association of β-lactamase genes with integron-carrying plasmids. Many β-lactamase genes within the integron have been reported, like bla_{CMY}, bla_{CTX-M}, and bla_{OXA} [17,18]. Moreover, integrons were also reported to be associated with fluoroquinolone resistance [19]. We noticed occurrence of integrons (as detected by amplifying 5'CS-3'CS region) in 55.20% enterobacterial isolates. However, the presence of integrons from community and nosocomial environment has been frequently reported [20-22]. Only 22% and 15% of isolates were found to carry Class 1 integrons in a Swiss [23] and Australian study [24] respectively. Shaheen, et al. reported the occurrence of Class 1 and class 2 integrons in 27% and 2% isolates respectively [25]. Bhattacharjee, et al. observed that among 63 ESBL-producing *K. pneumoniae* isolates, 58 (92%) carried Class 1 integrons and 6 isolates were found to harbour Class1 as well as Class 2 integron [26].

Sul-1 was reported to be present in 3'CS [27], but we also observe 14 isolates (out of 69) that showed amplification for 5'CS-3'CS region but did not show presence of *Sul-1* (when detected by PCR). Similar type of results was observed by Jin & Ling [28]. It was suggested by Jin & Ling that using sulfamethoxazole resistance as a marker for integrons

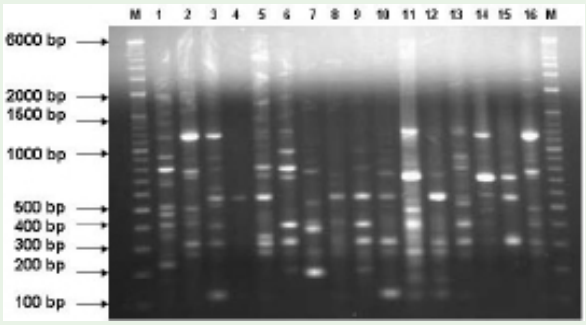

Figure 2c: RAPD profile of *E. coli* isolates obtained from Orthopaedics wards.

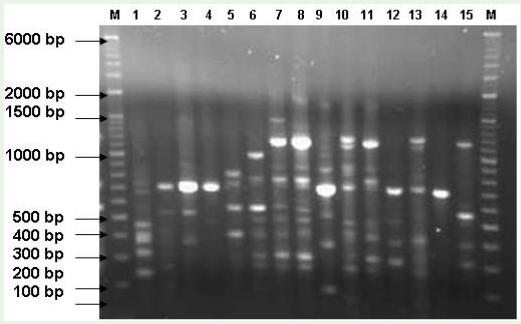

Figure 3: RAPD profile of *Klebsiella pneumoniae* isolates obtained from different wards.

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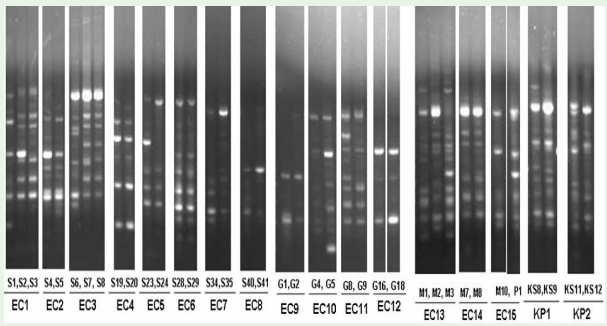

Figure 4: Representative Clusters of *E. coli* and *K. pneumoniae* isolates obtained from various wards.

could either misses the isolates or overestimates the prevalence of integrons. Insertion of *dfrA12-orfF-aadA2* or *orfF* leads to excision of *qacEΔ1* and *Sul-1* in 3'CS and hence integron without *Sul-1* gene were observed. Such excision has been reported in previous studies in some environmental bacteria [29]. We also noticed 22 isolates that were integron-negative but were found to harbor *Sul-1* gene. It may be possible that the *Sul-1* gene detected in these isolates was present in the second copy of 3'CS i.e. 3'CS2 which was not detected by integron PCR as we have used the reverse primer that is specific for first copy of 3'CS. Occurrence of non-classic integrons in *E. coli* isolates has been also reported [4,7,23,30-32].

The frequency of class 1 integrons could be underestimated in the literature as most studies are performed by PCR using specific primers designed in the 5'CS-3'CS. According to Saenz, et al. [32], some markers (absence of *qacEΔ1-Sul-1* and the presence of *qacH*, *Sul3* & *cmlA* genes) could be used to envisage the presence of non-classic integrons. Deletion/replacement of the typical *qacE1-Sul-1* sequence may result in the integration of more stable and efficiently expressed genes [33]. Laverstein-van Hall, et al. [34] noticed a significant association of multiple resistance genes with the presence of an integron and that the transfer of integron among bacterial species plays an important role in the development of multiple antibiotics resistance.

In our study isolates, single band was observed at variable position when PCR was performed by using primers specific for 5'CS and 3'CS. Similar type of results were observed by Peng, et al. [35] as they reported amplicons ranging from 0.7 to 3.0 kb obtained by PCR amplification of gene cassette region by using 5'CS-3'CS primers. The resistance genes of 1.0 kb (*aadA7*), 1.6 kb (*dhfrI-aadA1*), and 2.4 kb (*folA-catB3-aadA5*) class 1 integrons have also been reported [36,37]. It has been suggested that the deletion in the 3'CS segment or acquisition of different antibiotic resistance genes results in differences observed in molecular weight of amplicons.

It was evident from RAPD-typing that most patients in our hospital were infected with different clades of organisms, thereby demonstrating clonal diversity among isolates suggesting horizontal transmission of *bla* genes. RAPD-typing demonstrated that untypability of isolates is increasing. However, few isolates from gynaecology, surgery and orthopedics wards displayed similar banding patterns. Most probably, the same clone is circulating in these wards as the above described wards are sharing the same building block in our hospital and the chances of cross contamination increases.

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