

Horizontal Transfer of Antimicrobial Resistance by Extended-Spectrum β Lactamase-Producing *Enterobacteriaceae*

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ABSTRACT

Background: The purpose of this work was to study the acquisition of new antibiotic-resistant genes carried by extended spectrum β -lactamase (ESBL)-producing *Enterobacteriaceae* via horizontal transfer to understand their rampant spread in the hospitals and in the community.

Materials and Methods: A retrospective analysis of 120 ESBL screen-positive isolates of *Escherichia coli* and *Klebsiella pneumoniae*, which were subjected to antimicrobial susceptibility testing, was carried out. The Double Disc Synergy Test (DDST) and Inhibitor-Potential Disc Diffusion Test (IPDD) were employed for confirmation of ESBL activity. The transferability of the associated antibiotic resistance for amoxicillin, amikacin, gentamicin, cefotaxime and ceftriaxone was elucidated by intra- and intergenus conjugation in *Escherichia coli* under laboratory as well as under simulated environmental conditions. Transformation experiments using plasmids isolated by alkaline lysis method were performed to study the transferability of resistance genes in *Klebsiella pneumoniae* isolates.

Results: ESBL production was indicated in 20% each of the *Escherichia coli* and *Klebsiella pneumoniae* isolates. All the ESBL isolates showed co-resistance to various other groups of antibiotics, including 3GC antibiotics, though all the isolates were sensitive to both the carbapenems tested. Conjugation-mediated transfer of resistance under laboratory as well as environmental conditions at a frequency of $3-4 \times 10^{-5}$, and transformation-mediated dissemination of cefotaxime and gentamicin resistance shed light on the propensity of ESBL producers for horizontal transfer.

Conclusions: The transfer of resistant markers indicated availability of a large pool of resistance genes in the hospital setting as well as in the environment, facilitating long-term persistence of organisms.

Keywords: ESBL, horizontal transfer, conjugation, co-resistance, transformation

INTRODUCTION

Hospital-acquired bacterial infections may dominate the headlines, but most infections occur in the community. Indeed, 80% of the antibiotic prescribing takes place in the community – in local practices, daycare centers and long-term care facilities such as nursing homes and rehabilitation centers. Most patients hospitalized in the Intensive Care Units after being discharged continue to carry Extended Spectrum β -lactamase (ESBL) producing *Enterobacteriaceae* over prolonged periods. Continued carriage of such strains

may contribute to their extrahospital propagation.^[1]

In recent years, resistant bacteria have been isolated from apparently non-selective environments, including plants, coastal and estuarine environments, deep ocean water and sediments and drinking water.^[2] Organisms resistant to naturally occurring and human-modified antibiotics were detected in 22 rivers in the United States of America. A large proportion of the resistant organisms were ESBL producers, and many were found to contain plasmids with resistance traits.^[3] Resistance to additional classes of antibiotics has also been noted among ESBL-producing *Escherichia coli* and *Klebsiella* species (ESBL-EK) as many of these additional resistance genes are encoded on the ESBL-associated plasmid. The acquisition of resistance genes has not decreased the pathogenicity or virulence of *Klebsiella* species and *Escherichia coli*. Multidrug-resistant (MDR) ESBL-EK isolates (i.e., those resistant to multiple other antibiotics or antibiotic classes in

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addition to the oxyimino β -lactams) thus pose significant therapeutic challenges, even greater than those of ESBL-EK, further curtailing the number of drugs useful against these bacteria. Additionally, recently reported carbapenem-resistant ESBL-EK isolates are of paramount concern because of the scarcity of effective therapies for infections with such organisms.^[4]

Resistance genes can be spread far wider than once believed by horizontal gene transfer mechanisms like conjugation, transformation and transduction. Such gene transfer mechanisms allow mobilization of specific DNA fragments from one region to another, from plasmids to plasmids, from chromosome to chromosome and between plasmids and chromosomes. Plasmid-mediated diffusion of β -lactamases is of great concern and contributes to the enormous spread of this kind of enzyme throughout the microbial world.^[5] Considering the co-resistance of ESBLs with various other antibiotics and the extraordinary propensity of ESBL producers for horizontal gene transfer, the present work was aimed at studying the transfer of resistance among Gram negative bacteria like *Escherichia coli* and *Klebsiella pneumoniae* under laboratory as well as under simulated environmental conditions.

MATERIALS AND METHODS

Clinical isolates and antimicrobial susceptibility testing

A retrospective analysis of susceptibility patterns of 120 consecutive, non-repetitive ESBL screen-positive clinical isolates of *Escherichia coli* ($n = 70$) and *Klebsiella pneumoniae* ($n = 50$) was carried out. These isolates were obtained from a 100-bedded tertiary care hospital in Mumbai, India, during January 2007 to January 2008. The organisms were subjected to antimicrobial susceptibility testing by the disc diffusion method according to the Clinical and Laboratory Standards Institute (CLSI) guidelines on Mueller Hinton agar plates by using commercially available discs (Hi Media Laboratories, Mumbai, India) of amoxicillin (30 μ g), amikacin (30 μ g), gentamicin (30 μ g), trimethoprim (30 μ g), nalidixic acid (30 μ g), norfloxacin (10 μ g), ciprofloxacin (30 μ g), cefuroxime (30 μ g), cefoxitin (30 μ g), ceftazidime (30 μ g), cefotaxime (30 μ g), ceftriaxone (30 μ g), cefepime (30 μ g), imipenem (10 μ g), meropenem (10 μ g) and nitrofurantoin (300 μ g). The diameter of the zone of inhibition for each antibiotic was measured and interpreted as per the CLSI recommendations. The ESBL screen breakpoints criteria used were ≤ 22 mm for ceftazidime, ≤ 27 mm for cefotaxime and ≤ 25 mm for ceftriaxone. *Escherichia coli* ATCC 25922 as a negative quality control

strain and *Klebsiella pneumoniae* ATCC 700603 as a positive ESBL control (known to contain an SHV-type ESBL) were used for all the susceptibility testing studies as well as for the phenotypic confirmatory methods carried out for the detection of the ESBL producers.^[6]

Double disc synergy test

The Double disc synergy test (DDST) was performed to determine the synergy between a disc of amoxicillin/clavulanic acid (CA) (20/10 μ g) and 30 μ g discs of each ceftazidime and cefotaxime placed at a distance of 15 mm (center-to-center) from the amoxicillin/CA disc. Inoculated plates were incubated overnight at $36 \pm 1^\circ\text{C}$ for 24 h. Enhancement of the zone of inhibition between the clavulanate disc and any one of the β -lactam discs towards the amoxicillin/CA disc or lack of inhibition by either discs alone but inhibition of growth where the two antibiotics diffused together indicated the presence of ESBL.^[6,7]

ESBL confirmation by inhibitor-potential disc diffusion method

Inhibitor-potential disc diffusion (IPDD) was used to confirm the presence of ESBL-positive isolates by placing a disc of ceftazidime (30 μ g) and cefotaxime (30 μ g) alone and ceftazidime (30 μ g) and cefotaxime (30 μ g) in combination with CA (10 μ g), at least 20 mm apart from each other, on the Mueller Hinton agar plates. A 5-mm increase in zone diameter for either antimicrobial agent tested in combination with CA versus its zone when tested alone was taken as an indication of ESBL-producing isolates.^[6]

Intra-genus transfer of antibiotic resistance by conjugation

Intra-genus transfer of antibiotic resistance was studied using conjugation for all the ESBL-producing *Escherichia coli* isolates. A plasmid-free, streptomycin-resistant (F^- , Str^r) auxotrophic strain of *Escherichia coli* (*E. coli* AB 1157), showing sensitivity to all the antibiotics under study, was used as a recipient, while all the ESBL-producing *Escherichia coli* served as the donors. Overnight cultures were grown to an A_{540} of 0.1 (approximately 10^8 cells/mL). Five milliliters of each participating bacterial culture was mixed (1:1) in a test tube containing sterile Luria Bertani (LB) broth and incubated without shaking for 24 h at $36 \pm 1^\circ\text{C}$. The transconjugants were selected on the LB agar plates supplemented with streptomycin (100 μ g/mL) in addition to at least one of the following antibiotics, viz. amoxicillin, amikacin, gentamicin, cefotaxime and

ceftriaxone (30 µg/mL). In order to study the transfer of multiple resistance markers, plates with a series of combinations containing streptomycin with the remaining two to all the five antibiotics were used. The combinations included streptomycin with (i) amoxicillin and amikacin, (ii) amoxicillin, amikacin and gentamicin, (iii) amoxicillin, amikacin, gentamicin and cefotaxime and (iv) amoxicillin, amikacin, gentamicin, cefotaxime and ceftriaxone.^[7]

Inter-genus transfer of antibiotic resistance under laboratory and simulated environmental conditions by conjugation

Conjugation experiments were designed as described above to study the inter-genus transfer of the antibiotic resistance between the mating pairs consisting of ESBL-producing *Escherichia coli* and *Klebsiella pneumoniae* isolates displaying differing antibiotic-resistance patterns with respect to the antibiotics chosen. In order to study the inter-genus transfer in the environment, the experiment was carried out in sterile sewage and sea water instead of Luria Bertani broth and processed exactly in the same manner. After incubation, the bacteria were plated on the LB agar plates containing all the antibiotics mentioned above in order to select for the transconjugants.

Transformation mediated transfer of the antibiotic resistance in ESBL-producing *Klebsiella pneumoniae*

Transformation for the cefotaxime- and gentamicin-resistance markers was studied by using plasmid obtained from ESBL-producing *Klebsiella pneumoniae* isolates ($n = 10$) by the alkaline lysis method (minipreparation). Amplification of the plasmid was achieved by incubation of the individual 18 h old isolates (at $36 \pm 1^\circ\text{C}$ for 24 h) in LB broth containing chloramphenicol (30 µg). Plasmid DNA of the donors was transformed into chemically (CaCl_2) competent recipient cells.^[8] A *Klebsiella pneumoniae* isolate, obtained in the earlier study, showing sensitivity to gentamicin and cefotaxime but resistance to all the other antibiotics under study, was used as a recipient. Appropriate dilutions of transformants and control strains (untransformed) were streaked on LB agar plates containing cefotaxime (30 µg/mL) and gentamicin (30 µg/mL) added individually. LB plates supplemented with both gentamicin and cefotaxime added as a combination were used to detect the simultaneous transfer of both the markers.

RESULTS

In the present study, the overall prevalence of ESBL-

producing *Escherichia coli* (14/70) and *Klebsiella pneumoniae* (10/50) was 20% each by the DDST and as confirmed by the IPDD method. Ceftazidime detected 64.29% (9/14) ESBL-producing *Escherichia coli* and 70% (7/10) ESBL-producing *Klebsiella pneumoniae* while cefotaxime detected 71.43% (10/14) and 70.0% (7/10) ESBL-producing *Escherichia coli* and *Klebsiella pneumoniae*, respectively, by IPDD. Both the cephalosporins together identified 35.71% *Escherichia coli* and 40.0% *Klebsiella pneumoniae*.

The present investigation showed multidrug resistance among all the isolates used under the study (resistance to \geq three antibiotics). ESBL-producing *Escherichia coli* showed resistance to eight to 13 antibiotics tested while ESBL-producing *Klebsiella pneumoniae* showed resistance to six to 10 antibiotics tested. On the other hand, non-ESBL producers showed resistance to three to 11 antibiotics tested among both the isolates. All the isolates in the study, however, were sensitive to both the carbapenems tested. ESBL-producing *Escherichia coli* showed 100% resistance to amoxicillin, amikacin and nalidixic acid as seen in Table 1. Resistance of ESBL-producing *Escherichia coli* to third-generation cephalosporins used under study, viz. ceftazidime (85.7%), cefotaxime (71.4%) and ceftriaxone (78.6%), was notable. Resistance to gentamicin (92.9%) and nitrofurantoin (42.9%) was equivalent in ESBL and non-ESBL *Escherichia coli*. Eighty percent of the ESBL-producing isolates of *Klebsiella pneumoniae* showed resistance to amoxicillin, nalidixic acid, ciprofloxacin and ceftazidime, 50% to cefotaxime, 70% to ceftriaxone and 90% exhibited resistance to nitrofurantoin. However, non-ESBL-producing *Klebsiella pneumoniae* showed a higher resistance (75%) to cefepime compared with ESBL *Klebsiella pneumoniae* (20%).

Conjugation was used to elucidate intra- and inter-genus transfer of plasmid-borne resistance to a susceptible strain of *Escherichia coli* sensitive to all the antibiotics used in the study. The resistance was transferred to the recipient cells of *Escherichia coli* AB 1157 sensitive to the individual markers, i.e. amoxicillin, amikacin, gentamicin, cefotaxime and ceftriaxone, at a frequency of $3-4 \times 10^{-5}$. As the number of markers transferred increased from two to five, the frequency of transfer decreased from 10^{-5} to 10^{-7} . All the pairs of isolates assessed for the inter-genus transfer of the antibiotic resistance showed transfer of resistance. Among the four mating pairs tested, three pairs achieved two-way transfer of antibiotic-resistance markers under laboratory (LB broth) as well as under simulated environmental conditions (sewage and sea water), as shown in Table 2.

In the present study, plate assay was carried out to demonstrate the natural transformability of all the ten

Table 1: Resistance of ESBL-producing and non-producing *Escherichia coli* and *Klebsiella pneumoniae* to various antibiotics

No. (%) resistant to antibiotics	Am	Ak	G	Tr	Na	Nx	Cf	Cu	Cn	Caz	Ce	Ci	Cpm	Nf
<i>Escherichia coli</i>														
ESBL (n = 14)	14 (100)	14 (100)	13 (92.9)	11 (78.6)	14 (100)	11 (78.6)	10 (71.4)	13 (92.9)	12 (85.7)	12 (85.7)	10 (71.4)	11 (78.6)	5 (35.7)	6 (42.9)
Non-ESBL (n = 56)	52 (92.8)	51 (91.1)	52 (92.8)	32 (57.1)	50 (89.3)	28 (50.0)	28 (50.0)	46 (82.1)	33 (58.9)	44 (78.6)	20 (35.7)	32 (57.1)	9 (16.1)	24 (42.9)
<i>Klebsiella pneumoniae</i>														
ESBL (n = 10)	8 (80.0)	7 (70.0)	7 (70.0)	6 (60.0)	8 (80.0)	5 (50.0)	8 (80.0)	7 (70.0)	5 (50.0)	8 (80.0)	5 (50.0)	7 (70.0)	2 (20.0)	9 (90.0)
Non-ESBL (n = 40)	30 (75.0)	22 (55.0)	25 (62.5)	20 (50.0)	20 (50.0)	17 (42.5)	17 (42.5)	16 (40.0)	15 (37.5)	26 (65.0)	20 (50.0)	24 (60.0)	30 (75.0)	21 (52.5)

Values in parentheses indicate percentage; Am – amoxicillin; Ak – amikacin; G – gentamicin; Tr – Trimethoprim; Na – nalidixic acid; Nx – norfloxacin; Cf – ciprofloxacin; Cu – cefuroxime; Cn – ceftoxitin; Caz – ceftazidime; Ce – cefotaxime; Ci – ceftriaxone; Cpm – ceftipime; Nf – nitrofurantoin

Table 2: Intergenous transfer of resistance by conjugation

Mating pair	Resistance pattern										Type of resistance transfer
	Before co incubation					After co incubation					
	Am	Ak	G	Ce	Ci	Am	Ak	G	Ce	Ci	
<i>E. coli</i> 64 + <i>K. pneumoniae</i> 35	R	R	R	S	R	R	R	R	R	R	Resistance for Ce transferred from <i>K. pneumoniae</i> 35 to <i>E. coli</i> 64 and resistance for Am, Ak and G transferred from <i>E. coli</i> 64 to <i>K. pneumoniae</i> 35
	S	S	S	R	R	R	R	R	R	R	
<i>E. coli</i> 43 + <i>K. pneumoniae</i> 13	R	R	R	R	R	R	R	R	R	R	Resistance for G and Ci transferred from <i>E. coli</i> 43 to <i>K. pneumoniae</i> 13
	R	R	S	R	S	R	R	R	R	R	
<i>E. coli</i> 26 + <i>K. pneumoniae</i> 11	R	R	R	R	S	R	R	R	R	R	Resistance for G transferred from <i>E. coli</i> 26 to <i>K. pneumoniae</i> 11 and resistance for Ci transferred from <i>K. pneumoniae</i> 11 to <i>E. coli</i> 26
	R	R	S	R	R	R	R	R	R	R	

Am – amoxicillin; Ak – amikacin; G – gentamicin; Ce – cefotaxime; Ci – ceftriaxone; R – resistant; S – sensitive

ESBL-producing *Klebsiella pneumoniae* isolates *in vitro*. In all the cases, transformants were obtained on LB agar plates, indicating the development of the competence and uptake of DNA, leading to recombination and thus, transformation. Both the markers were transferred at a low frequency of about $1-5 \times 10^{-7}$. Cefotaxime resistance was transferred in all the isolates while only three isolates showed transfer of gentamicin resistance. Two isolates showed simultaneous transfer of both the markers at a frequency of $1-3 \times 10^{-7}$.

DISCUSSION

In India, ESBL-producing strains of *Enterobacteriaceae* have emerged as a challenge in the hospitalized as well as in the community-based patients. In 2002, 68% of the Gram negative bacteria were found to be ESBL producers in a study from New Delhi, with 80% of *Klebsiella* being ESBL-producers.^[9] In the present study, the incidence of ESBL was, however, much lower, at 20%. Distinct regional variations have been detected in the incidences of ESBL-producing isolates, which can be attributed to the different patterns of antibiotic use.

Although the phenotypic confirmatory tests for the detection of ESBLs have proven reliable over many years at detecting the great majority of conventional ESBLs, the strains that are positive on the screening test but negative on the confirmation test (false-positive) or isolates that fail to indicate the presence

of ESBL in the screen test but are detected in the combination test (false-negative) need attention. In the present work, 56 isolates of *Escherichia coli* and 40 isolates of *Klebsiella pneumoniae* were screen positive but failed to show clavulanic acid inhibition effect (CAIE). This can be attributed to the additional resistance mechanisms possessed by the organisms that can mask the presence of ESBL activity. In particular, the emergence of plasmid-borne AmpC β -lactamases, which are not inhibited by CA in the members of the *Enterobacteriaceae*, is likely to explain at least some of the strains that have a positive screening test but a negative confirmation test. Other mechanisms include possible co-existence of AmpC with ESBLs, hyperproduction of ESBL, modification of outer membrane proteins, TEM and SHV β -lactamases that are no longer inhibited by CA due to mutations in the coding sequences, etc.^[10]

Outbreaks of ESBL-producing *Enterobacteriaceae* strains, often characterized by resistance to multiple drugs, including ciprofloxacin, gentamicin and aminoglycosides, have been reported. A study in 2005 reported an occurrence of 18.8% of MDR ESBL-EK isolates, demonstrating variable resistance to other antibiotics and antibiotic classes except imipenem. There was a high prevalence of resistance to fluoroquinolone, ceftipime and piperacillin-tazobactam. They found no association between MDR ESBL-EK and prior antibiotic exposure.^[11] A study in 2008 in Chandigarh with Gram negative ESBL-positive uropathogenic bacteria showed a high

degree of resistance to piperacillin (93.1%), amoxicillin–CA (93.4%), aztreonam (79.4%), cefepime (76.7%), ampicillin–sulbactam (76.7%) and ticarcillin (60.2%). Similarly, ESBL non-producers also showed a high degree of resistance to piperacillin and amoxicillin–CA (90.9% each), followed by cefepime (78%), aztreonam (78%) and ticarcillin (75.7%).^[12] Similar results were found in the present investigation. All the isolates showed sensitivity to both imipenem and meropenem. These findings are in accordance with the studies carried out by other workers.^[7,13] The high resistance of non-ESBL-producing *Klebsiella pneumoniae* is in accordance with the results reported in the literature.^[12] Naturally occurring resistance to expanded-spectrum cephalosporins can be mediated by overexpression of the chromosomal cephalosporinase or by the expansion of the substrate specificity of the AmpC β -lactamases.^[14]

As resistance plasmids are the major source of ESBL transmission, transferable elements conferring resistance to antimicrobials other than β -lactams travel on or alongside the ESBL-containing plasmids, yielding MDR bacteria. It is also possible that mechanisms other than, or in addition to, plasmid-mediated co-transfer of various resistance factors account for the phenomenon of co-resistance observed. Plasmid-mediated diffusion of β -lactamases is of great concern and contributes to the enormous spread of this kind of enzyme throughout the microbial world. About 60% of *Escherichia coli* strains are now considered to carry a TEM-1, TEM-2 or SHV-1 enzyme. Broad-spectrum β -lactamase derivatives and other ESBLs that appeared in the mid-1980s are increasing among *Escherichia coli* strains (and other species). The above mentioned genes can spread rapidly within a bacterial population and from one environment to another by sharing and exchanging genetic information. The reservoir of such enzymes might be the commensal microbiota of healthy individuals and patients undergoing antibiotic treatment. When the selective pressure of antibiotics is exerted, bacteria already have a large population of resistance genes available to them. This provides an environment where the development and spread of antibiotic resistance is likely to continue indefinitely, both due to the selective pressure of antibiotics and to the resistance already present in the population.^[5] A study of 100 healthy executives receiving a comprehensive health check at a tertiary care center in central Mumbai in 2004 showed the presence of 11% ESBL-producing *Escherichia coli* and *Klebsiella pneumoniae* in stool samples. None of the executives gave a history of hospitalization in the last year or history of antimicrobial drug consumption in the last 6 months.^[15] A study in Spain showed fecal carriage (4.4–66.6%) of ESBL-producing *Enterobacteriaceae* in samples from 19 of 61 food-borne outbreaks evaluated, suggesting that the community could act as a reservoir.^[16]

The ability of the plasmid DNA to transfer or be mobilized between different strains of the same species or between bacterial species has been demonstrated repeatedly. As more and more genome sequences are determined, it is becoming clear that inter-species transmission of the genetic information is pervasive among microorganisms and that it may occur at vast phylogenetic distances and change the ecological and pathogenic character of bacterial species. A study demonstrated transfer of ESBL-mediated resistance to cefotaxime along with transfer of gentamicin and other beta-lactam antibiotics.^[7] In a study conducted in France in 1996, mating between exponentially growing cultures of *Klebsiella pneumoniae* RIC and *Escherichia coli* K12 J53-2 resistant to rifampin or *Escherichia coli* K12 C600 resistant to nalidixic acid was performed. The transfer of β -lactam resistance determinants from donor cells was obtained at a frequency of 10^{-7} . Resistance markers to aminoglycosides, tetracyclines, chloramphenicol, trimethoprim and sulfonamides were co-transferred.^[17]

In a study in China, transfer of resistance to ceftazidime, cefotaxime, ceftriaxone, gentamicin, amikacin, ciprofloxacin, aztreonam, ceftiofloxacin and ticarcillin/CA and intermediate resistance to piperacillin/tazobactam, cefoperazone/sulbactam and cefepime was achieved from the clinical isolates of *Klebsiella pneumoniae* to *Escherichia coli* C600 by conjugation with a transfer frequency of 10^{-6} to 10^{-7} .^[18] The results of this study also reiterated the fact that difference in the genera did not affect the transfer of the resistance in any way.

A pool of resistance is developing in the non-pathogenic organisms found in humans, animals and the environment. In recent years, resistant bacteria have been isolated from apparently non-selective environments, including plants, coastal and estuarine environments, deep ocean water and sediments and drinking water. The release of the non-disinfected wastewaters into the marine/aquatic environment, a common worldwide practice, in underdeveloped as well as in highly developed countries, exerts enormous pressure on these environments. Both the resistant microorganisms and the residues of antibiotics administered to humans and animals reach the sewage systems in urine or feces in the form of either the parent compound or the degraded metabolites depending on the pharmacology of the specific antibiotic. Waste effluent from hospitals contains high numbers of resistant bacteria and antibiotic residues between a concentration of 1 and 100 $\mu\text{g/L}$, which has a potential to select for antibiotic resistance. The pathogenic organisms serve as a source from which non-pathogens can acquire genes conferring resistance and in turn, they can become resistant by acquiring the genes from the pathogens discharged into the environment. Thus, dissemination of the resistant bacteria

is not only a problem of the resistant pathogens themselves but also availability of the resistance genes to the pathogens via horizontal gene transfer in practically every environment.^[2] The conjugal transfer of plasmids in various environments has been observed by various workers. A study carried out on the marine environment of Mumbai demonstrated not only presence of ESBL-producing *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas* and *Salmonella paratyphi* B in the coastal waters of Mumbai but also demonstrated transfer of antibiotic resistance in sea water as well as in biofilms.^[19]

An increasing body of evidence points at natural genetic transformation as an important mechanism of the horizontal exchange of genes. Natural genetic transformation is a gene transfer process where the bacteria can pick up the naked DNA from their environment. The DNA may come from a variety of sources, but the most frequent is remnants from dead bacterial cells. The process involves the binding of the exogenous DNA to specific cell surface receptors, then the DNA is transported across the membrane and one strand of the DNA is digested away. The DNA that enters the cell is thus single stranded. As long as the incorporated DNA is sufficiently homologous to the host DNA, recombination occurs and the new DNA replaces a strand of the host DNA. If the new DNA is of a different allelic nature than the host DNA, a gene conversion event can occur.^[5] A study carried out on isolates of *Enterobacteriaceae* from 43 hospitals in the UK during 1990–1991 demonstrated transfer of resistance from the plasmid DNA extracted from three isolates of *Klebsiella pneumoniae* by transformation.^[20] Another study in France also showed co-transfer of chloramphenicol, tetracycline and sulfonamide resistance with the CTX-M-1 enzyme using transformation experiments.^[1]

The results of the study reiterated the fact that the well-established clones enhance their resistance phenotype by the acquisition of the new resistant genes via gene capture genetic units such as plasmids. The intra- and inter- generic transfer of resistant markers in the laboratory as well as in the environment points towards the availability of a large pool of resistance genes in the hospital setting as well as in the environment thus facilitating the long-term persistence of organisms in the selective environments. In this scenario, a multiclonal population structure of bacteria corresponds to a collection of different strains sharing resistance genes carried by horizontally transferred genetic structures.

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