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Transfer of Antimicrobial Resistance Genes from Clinical Serotypes of Enteropathogenic *Escherichia coli* to *E. coli* K12

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Abstract: ***Background*:** It has been shown that about 60% of the multiple-drug-resistant coliform isolates transfer their plasmids to laboratory strains of *E. coli*, *Salmonella*, or *Shigella*. Mostly, the actual transfer frequencies were not determined, nor were the abilities of fecal and environmental strains of *Escherichia coli* and enteric pathogens to receive the plasmids considered**.** ***Materials and Methods*:** A total of 614 children feces samples (363 diarrhetic from children hospital and 251 healthy children from Mosul nurseries). All children were under three years of age. Fecal samples were transported to research laboratory using nutrient broth as transport medium for rectal swabs. EPEC identification, serotyping and antimicrobial resistance genes transfer were carried out**.** ***Results*:** The R determinants transfer from clinical isolates of *E. coli* to E.coli K12 and the number were 4, 15 and 7 for tetracycline, trimethoprim and ampicillin and their frequency range of transfer was 3.1 x 10^{-3} -5 x 10^{-2}, 5.2 x 10-4 – 2.1 x 10^{-2} and 5.2 x 10^{-4} – 7.5 x 10^{-3} for tetracycline, trimethoprim and ampicillin respectively. The frequency range of R resistance determinant transfer for lead, zinc and mercury was 2.6 x 10^{-4} to 8.3 x 10^{-2}, 1 x 10^{-3} to 6.3 x 10^{-3} and 1.8 x10^{-3} to 2.5 x 10^{-2} respectively. It was also noticed that the frequency of resistance transfer of disinfectants for receipt ranged between 1.3 x 10^{-3} to 1.2 x 10^{-1} which was the highest among all antimicrobial agents tested. ***Conclusions*:** Tetracycline resistance determinants exhibited the highest conjugative transfer frequencies, indicating that TE-associated R plasmids are transferred more efficiently than those carrying TMP or AMP resistance. Recipient frequencies were consistently lower than donor frequencies, indicating transfer inefficiency and/or reduced establishment of resistance determinants in the recipient strain (*E. coli* K12). Forty four serotypes were used for metal resistance transfer and only 29, 2 and 6 serotypes showed transfer for lead, zinc and mercury respectively. The present study revealed that the cetrimide transfer frequency from clinical isolates to E, coli K12 and 12 out of 13 serotypes showed resistance transfer with range from 3.4 x 10^{-4} to 4.9 x 10^{-2}; 2 of them were of O125 serotype of donors.

Keywords: Children, Diarrhea, EPEC, Serotypes, Antimicrobials Resistance, Gene Transfer.

Research Paper

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INTRODUCTION

The basis for concern is that multiple-drug resistant coliforms have been isolated from sewage, its P receiving waters, and sludge-dumping areas, where they comprised between 1 and 50% of the coliform population [1-4]. In addition, it has been shown that about 60% of the multiple-drug-resistant coliform isolates transfer their plasmids to laboratory strains of *E. coli*, *Salmonella*, or *Shigella* [5-8]. However, in most of these studies, the actual transfer frequencies were not determined, nor were the abilities of fecal and environmental strains of *E. coli* and enteric pathogens to receive the plasmids considered. These factors are important in the assessment of risk. The present investigation was initiated to reexamine the question of water-bome dissemination of R plasmids with emphasis on these two considerations. It was found that the probability that an R plasmid from the ingested R+ *E. coli* will be transferred to a resident E. coli or a subsequently ingested enteric pathogen could be

increased if the R+ E. coli colonized the bowel of the individual and thereby increased its numbers. Plasmid transfer between *E. coli* strains can occur in the gut [9], even in the absence of antibiotics [10-12], although this is a rare phenomenon [13]. Usually, though, more than 106 bacteria must be ingested in order for colonization to occur [14], and even when 1010 viable organisms are ingested, colonization by R-plasmid- bearing strains is poor [15]. Furthermore, if R-plasmid transfer between an ingested R+ coliform and a resident E. coli strain does occur, there is a question as to how long the R+ transconjugant will remain in the bowel [16-19]. This period of time will influence the probability that the R+ bacteria will transfer resistance to a subsequently ingested enteric pathogen. Diarrheagenic *E. coli* is currently categorized into a limited number of groups, primarily based on the presence of specific virulence genes, and is classified under a single pathogenic variant (pathovar). EPEC strains that possess the Locus of Enterocyte Effacement (LEE) region and Bundle-Forming Pilus (BFP) are referred to as typical EPEC (tEPEC), while those that lack the BFP are designated as atypical EPEC (aEPEC) [20-23]. In 28 % of the resistant strains R factors transmissible to *E. coli* K12 W 3132 could be demonstrated. This does not necessarily mean that R factors were not present in 72 %. It is well known that R factors may be segregated and accordingly loose their transmissibility. The use of more than one recipient strain might have increased the possibility to demonstrate R factors. *E. coli V\T* 3132, however, was shown to be a competent recipient of some R factors, since the frequency of transfer in some experiments was higher than 10-2•. In the meantime, ETEC is identified at the molecular level by the presence of heat-labile (LT) or heat-stable (ST) enterotoxins, along with a variety of accessory virulence factors, including the *eae* autotransporter [24]. These canonical destructiveness variables are frequently encoded on plasmids or other mobile components within the segregates from each of these pathovars [25-28]. Some factors, including heavy metals and antibacterial biocides, can co-select for antibiotic-resistant strains via cross-resistance (that is, via the same mechanism) or co-resistance (that is, via genetically linked mechanisms) [29-31]. For example, *Enterococcus xiangfangensis* from the intestine showed resistance not only to β-lactam, quinolone, aminoglycoside and sulfonamide antibiotics but also to an unusually high number of plasmid-based heavy metal resistance gene clusters, such as copper, mercury and cadmium [16]. Gram-negative bacteria increased significantly after the addition of heavy metals and were more prone to receiving resistant plasmids from donors [32, 33]. The increasing use of antibiotics and heavy metals creates a selective pressure for the survival of bacteria by resistance through mutations or plasmid acquisition [34]. Heavy metals and antibiotic resistance genes were found on plasmids together, which transferred to microorganisms [35]. The exposure to low concentrations of heavy metals may select for the resistance of antibiotics and adaptation of bacteria [36, 37]. Antibiotic multiple resistance (AMR) continues to persist despite the reduction in usage of antimicrobial drugs on European farms and an increase in the application of biosecurity practices [38, 39]. Evidence of co- and cross-resistance between antibiotics and other antimicrobials, such as disinfectants, gives rise to concerns over the efficacy of C and D measures [40, 41]. Co-resistance may occur when AMR genes and disinfectant tolerance genes (such as those regulating efflux or biofilm formation) are located on the same mobile genetic element, such as a plasmid [42, 43]. On the other hand, cross-resistance occurs when the same mechanism, for example, a gene involved in efflux, leads to altered susceptibility against both antibiotics and disinfectants [44, 45]. Consequently, AMR bacterial pathogens may harbour the potential for tolerance to disinfectants, thus influencing the efficacy of C and D practices. The application of disinfectants throughout human and animal sectors, and in environmental facilities such as pollution management facilities, makes this a One Health concern [46-48].

MATERIALS AND METHODS

Patients

This study was carried out in hospital of Children hospital and nursey of Mosul city, Iraq. 602 infants were included and they were 351 infants with diarrheic diseases and 251 infants from nursery. The acceptance for participation in the present study was taken from all the participants families whose native language is Arabic. They were not mentally retarded and they were completely healthy considering hearing and speaking.

Sampling

Rectal swabs were taken from each infant. Swabs were rinsed with sterile nutrient broth as a transport media. 351 samples of paediatric hospital patients and 251 swabs were from nursery infants [4-50].

Isolation

All samples were inoculated on MacConkey agar (Oxoid). Inoculated plates were incubated at 37 ^{0}C for 24 hours [51, 52].

Identification of Escherichia Coli

The purified isolates of suspected *E.coli* were conventionally. All plates of MacConkey agar were examined for the presence of lactose fermenting colonies. One to three suspected colonies were selected from each plate and tested for indole, acid and gas production from lactose and for ability of growth at 44 ^{0}C.These suspected *E. coli* were further identified by IMViC tests; H_2S production; fermentation of sucrose, maltose, xylose, rhamnose, sorbose , raffinose, sorbitol, dulcitol and salicin; decarboxylation of ornithine, arginine and lysine [4-55].

Serotyping

BioMerieux (BioMerieux, Laboratory Reagents and Products, France) slide agglutination technique was utilized for identification of different groups of serotypes of enteropathogenic *Escherichia coli* as mentioned by the manufacturer [56].

Determination of Antibiotic Resistance Transfer

Ten tetracycline-resistant strains of *Escherichia coli* were selected and subjected to antibiotic resistance transfer experiment as donors before and after the disinfectant passages. *Escherichia coli* J53.2 (F− pro met rifr) was used as a recipient strain, which was kindly provided by Dr. K.J. Towner (University of Nottingham, UK) and the procedure performed as follows: donors and recipients were grown in 10mL nutrient broth at 37°C for 6 h. Three ml of the resistant donor broth was then mixed with 3 mL of the recipient broth and the mixtures incubated at 37°C for 18 h. Then, 10−1 and 10−2 dilutions of the mating mixtures were prepared in 1:4 Ringer's solution, and0.1 mL of these, together with undiluted mating mixture, were seeded onto selective plates of MH agar containing100 µg mL−1 rifampicin and 50 µg mL−1 tetracycline [1-57].

Determination of Resistance Transfer to Heavy Metals

The methods used for heavy metals almost the same of that utilized in antibiotic resistance transfer and the only difference was the concentrations incorborated in Mueller-Hinton agar that resisted by clinical isolates. These concentrations were 1000, 200, 100 and 10 µg/ml for lead, zinc, cadmium and mercury respectively [37].

Determination of Resistance Transfer to Disinfectants

The methods used for disinfectants almost the same of that utilized in antibiotic resistance transfer and the only difference was the concentrations incorporated in Mueller-Hinton agar that resisted by clinical isolates. The concentrations were 100, 50 and 2 µg /ml for chloroxylenol, cetrimide and chlorhexidine respectively [46].

Statistical Analyses

All statistical analyses were performed using IBM SPSS Statistics for Windows, Version 26.0 (IBM Corp., Armonk, NY, USA). Descriptive statistics such as means, standard deviations, and frequency distributions were computed to summarize the data. A paired sample t test correlation coefficient (R) and coefficient of determination (R2) were calculated [58-60].

RESULTS

Antibiotic Resistance Transfer

Three antibiotics were selected for this experiment and these were tetracycline, trimethoprim and ampicillin and number of resistant serotypes to these antibiotics were selected which were 13, 22 and 9 respectively (Table 1). The R determinants transfer from clinical isolates of *Escherichia coli* to E.coli K12 and the number were 4, 15 and 7 for tetracycline, trimethoprim and ampicillin and their frequency range of transfer was 3.1×10^{-3} -5 x 10^{-2} , 5.2 x 10-4 – 2.1 x 10^{-2} and 5.2 x 10^{-4} – 7.5 x 10^{-3} for tetracycline, trimethoprim and ampicillin respectively. Pearson correlation between donor and recipient transfer frequencies (r) value was 0.396. Tetracycline resistance determinants exhibited the highest conjugative transfer frequencies, indicating that TE-associated R plasmids are transferred more efficiently than those carrying TMP or AMP resistance. Recipient frequencies were consistently lower than donor frequencies, indicating transfer inefficiency and/or reduced establishment of resistance determinants in the recipient strain (*E. coli* K12). Ampicillin determinants demonstrated the lowest transfer frequencies, suggesting reduced dissemination potential (Figure 1).

Table 1: Frequency R determinant of antibiotics resistance from donor *Escherichia coli* to receipt *Escherichia coli* K12

Antibiotic*	Isolate code	serotype	Donor transfer frequency	Receipt transfer frequency
Tetracycline (TE)	352H	O86	1.3×10^{-2}	3.1×10^{-3}
	128N	O125	2×10^{-2}	1.3×10^{-2}
	9H	O125	3.1×10^{-3}	1.6×10^{-3}
	297H	O126	5×10^{-2}	6.3×10^{-3}
Trimethoprim (TMP)	361H	O86	1.3×10^{-2}	7.5×10^{-3}
	43H	O111	1.3×10^{-2}	2.3×10^{-2}
	148H	O111	1.7×10^{-2}	1×10^{-2}
	188H	O111	6.3×10^{-3}	2.5×10^{-3}
	149H	O114	8.3×10^{-3}	5×10^{-3}
	218H	O114	5.2×10^{-4}	7.5×10^{-3}
	111N	O125	7.1×10^{-2}	1×10^{-2}
	200H	O125	1×10^{-2}	1.3×10^{-2}
	121N	O126	9.6×10^{-4}	2.5×10^{-3}
	214H	O126	8.3×10^{-3}	5×10^{-3}
	230H	O126	2.5×10^{-3}	2.5×10^{-3}
	143N	O142	2.3×10^{-3}	5×10^{-3}
	181H	O142	6.3×10^{-3}	5×10^{-3}

Antibiotic*	Isolate code	serotype	Donor transfer frequency	Receipt transfer frequency
	325H	O142	2.1 X 10^{-2}	1.3 X 10^{-2}
	265H	OI+II+III	1.8 X 10^{-3}	2.5 X 10^{-3}
Ampicillin (AMP)	52H	O111	8.3 X 10^{-4}	1.3 X 10^{-3}
	51H	O119	3.1 X 10^{-3}	1.3 X 10^{--3}
	362H	O125	5.2 X 10^{-4}	1.2 X 10^{-3}
	220H	O126	6.3 X 10^{-3}	2.5 X 10^{-3}
	312H	O142	7.5 X 10^{-3}	3.8 X 10^{-3}
	216H	O142	1.9 X 10^{-3}	2.5 X 10^{-3}
	116H	OI+II+III	1.3 X 10^{-3}	1.3 X 10^{-3}

*Number of serotypes tested= Tetracycline, 12; Trimethoprim, 22; Ampicillin, 9. H, Hospital; N, Nursery

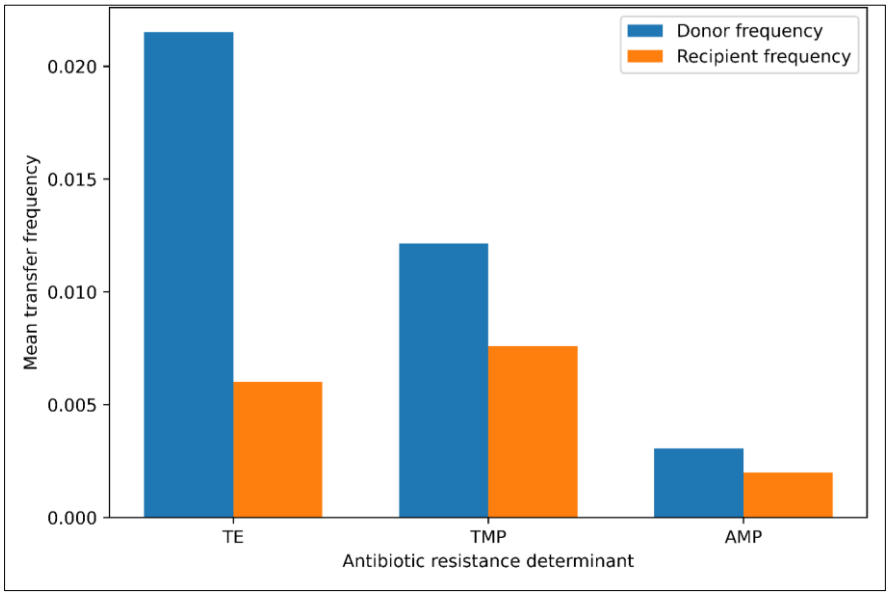

Figure 1: Mean donor and recipient transfer frequencies of antibiotic resistance determinants transferred from donor *Escherichia coli* isolates to recipient *E. coli* K12

Heavy Metal Resistance Transfer

Forty four serotypes were used for metal resistance transfer and only 29, 2 and 6 serotypes showed transfer for lead, zinc and mercury respectively (Table 2). The frequency range of R resistance determinant transfer for lead, zinc and mercury was 2.6 x 10^{-4} to 8.3 x 10^{-2}, 1 x 10^{-3} to 6.3 x 10^{-3} and 1.8 x10^{-3} to 2.5 x 10^{-2} respectively (Table 2). Forest plot analysis revealed substantial variation in the conjugative transfer frequencies of heavy-metal resistance determinants. Cadmium- and mercury-associated determinants showed the highest mean transfer frequencies, whereas zinc-associated determinants exhibited the lowest transfer efficiencies. Lead resistance determinants demonstrated intermediate transfer rates with considerable variability among isolates. Overall, donor transfer frequencies exceeded recipient transfer frequencies, indicating incomplete establishment of transferred determinants in recipient *E. coli* K12 cells. The observed differences suggest that certain heavy-metal resistance determinants possess enhanced dissemination potential and may contribute more significantly to the environmental spread of mobile resistance elements (Figure 2).

Table 2: Frequency R determinant of heavy metals resistance from donor *Escherichia coli* to receipt *Escherichia coli* K12

Heavy metal*	Isolate code	serotype	Donor transfer frequency	Receipt transfer frequency
Lead (Pb)	108N	O25	5 X 10^{-3}	2.1 X 10^{-3}
	59N	O86	1.8 X 10^{-3}	7.4 X 10^{-4}
	222N	O86	4.7 X 10^{-3}	2.2 X 10^{-3}
	43H	O111	7.8 X 10-2	6.6 X 10^{-3}
	148H	O111	7.2 X 10^{-3}	1.5 X 10^{-3}
	149H	O114	7.9 X 10^{-3}	2.1 X 10^{-3}
	1N	O119	1.5 X 10^{-3}	1.5 X 10^{-3}
	17N	O119	6.3 X 10^{-3}	1.5 X 10^{-3}

Heavy metal*	Isolate code	serotype	Donor transfer frequency	Receipt transfer frequency
	165H	O119	1.6×10^{-3}	7.4×10^{-4}
	51H	O119	6.3×10^{-3}	7.4×10^{-4}
	362H	O125	1.8×10^{-3}	7.4×10^{-4}
	111N	O125	1.8×10^{-3}	5.1×10^{-3}
	128N	O125	7.8×10^{-3}	4.4×10^{-3}
	9H	O125	2.5×10^{-2}	4×10^{-2}
	95H	O125	5×10^{-3}	1.5×10^{-3}
	200H	O125	1.7×10^{-2}	4.4×10^{-3}
	121N	O126	8.7×10^{-3}	1.5×10^{-2}
	214H	O126	3.3×10^{-3}	4.4×10^{-3}
	220H	O126	2.5×10^{-3}	7.4×10^{-4}
	297H	O126	1.7×10^{-2}	2.9×10^{-3}
	12N	O127	1.8×10^{-3}	7.4×10^{-4}
	132N	O127	2.6×10^{-2}	4.3×10^{-2}
	115N	O142	1.6×10^{-3}	1.5×10^{-3}
	181H	O142	4.7×10^{-3}	1.5×10^{-3}
	312H	O142	7.1×10^{-3}	7.4×10^{-4}
	316H	O142	2.1×10^{-3}	1.5×10^{-3}
	116N	OI+II+III	7.8×10^{-3}	1.5×10^{-3}
	124N	OI+II+III	7.6×10^{-4}	1.5×10^{-3}
	152N	OI+II+III+IV	7.1×10^{-3}	7.4×10^{-4}
Zinc (Zn)	128N	O125	6.3×10^{-3}	7.4×10^{-4}
	316H	O142	1×10^{-3}	7.4×10^{-4}
Cadmium (Cd)	148H	O111	3.1×10^{-3}	2.2×10^{-3}
	128N	O125	7.5×10^{-2}	2.1×10^{-3}
	200H	O125	2.1×10^{-3}	7.4×10^{-4}
	42N	O142	5×10^{-3}	1.5×10^{-3}
	115N	O142	1.9×10^{-3}	1.5×10^{-3}
	116N	OI+II+III	2.8×10^{-3}	1.5×10^{-3}
Mercury (Hg)	9H	O125	1.5×10^{-2}	2.4×10^{-3}

*Number of serotypes tested= Lead, Cadmium, Zinc, 44 each; Mercury,5. N, Nursery, H, Hospital.

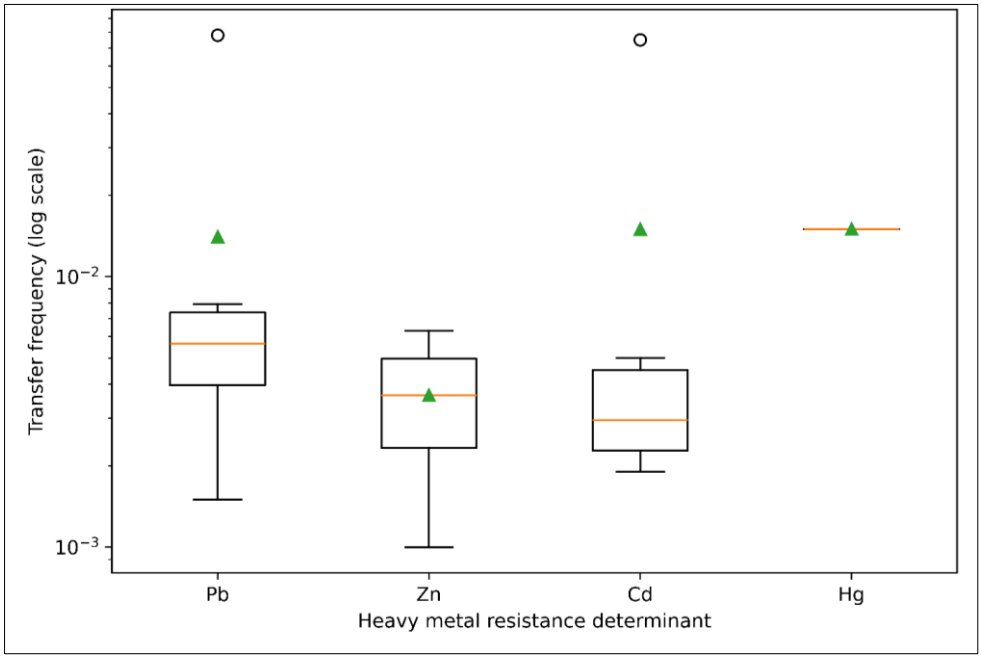

Figure 2: Forest plot of donor-to-recipient transfer frequencies of heavy-metal resistance determinants among *Escherichia coli* serotypes

Disinfectant Resistance Transfer

The present study revealed that the cetrimide transfer frequency from clinical isolates to E, coli K12 and 12 out of 13 serotypes showed resistance transfer with range from 3.4 x 10^{-4} to 4.9 x 10^{-2} ; 2 of them were of O125 serotype of donors. It was also noticed that the frequency of resistance transfer for receipt ranged between 1.3 x 10^{-3} to 1.2 x 10^{-1} which was the highest among all antimicrobial agents tested. The forest plot demonstrated variability in the transfer frequencies of disinfectant resistance determinants among *Escherichia coli* isolates. The 95% confidence intervals indicated that cetrimide resistance determinants exhibited the greatest variability in transfer frequency, whereas chloroxylenol resistance determinants showed the narrowest distribution. The wider confidence intervals observed for cetrimide and chlorhexidine suggest substantial heterogeneity among serotypes in their conjugative transfer capacity. Overall, the confidence intervals support the conclusion that disinfectant resistance determinants differ in their dissemination potential, with cetrimide-associated determinants showing the highest transfer efficiency.

Table 3: Frequency R determinant of disinfectants resistance from donor *Escherichia coli* to receipt *Escherichia coli* K12

Disinfectant*	Isolate code	serotype	Donor transfer frequency	Receipt transfer frequency
Cetrimide	51N	O86	2.4 X 10^{-3}	7.5 X 10^{-2}
	222N	O86	1 X 10^{-2}	1.5 X 10^{-2}
	1N	O119	1.3 X 10^{-3}	3.8 X 10^{-3}
	12N	O119	5.4 X 10^{-2}	2.8 X 10^{-2}
	165N	O119	5.4 X 10^{-4}	1.2 X 10^{-3}
	95H	O125	2.8 X 10^{-2}	5 X 10^{-2}
	287H	O125	8.3 X 10^{-4}	1.2 X 10^{-3}
	12H	O127	8.2 X 10^{-3}	1 X 10^{-2}
	132N	O127	4.9 X 10^{-2}	1.2 X 10^{-1}
	42N	O142	3.4 X 10^{-4}	2.5 X 10^{-3}
	115N	O142	7.4 X 10^{-4}	1.3 X 10-3
	152N	O+I+II+III+IV	1.9 X 10^{-3}	2.5 X 10^{-3}
Chlorhexidine	111N	O125	1.5 X 10^{-2}	4.4 X 10^{-3}
	297H	O126	1.3 X 10^{-2}	2.9 X 10^{-2}
	116N	OI+II+III	2.8 X 10^{-2}	1.5 X 10^{-2}
	265H	OI+II+III	6 X 10^{-4}	7.4 X 10^{-4}
Chloroxylenol	111N	O125	2.5 X 10^{-3}	7.4 X 10^{-4}
	128N	O125	6.3 X 10^{-3}	7.4 X 10^{-4}
	9H	O125	4.6 X 10^{-4}	7.4 X 10^{-4}
	152N	OI+II+III+IV	6.3 X 10^{-3}	1.5 X 10^{-5}

*Number of serotypes tested= Cetrimide,13; Chlorhexidine,5; Chloroxylenol, 44. N, Nursery; H, Hospital.

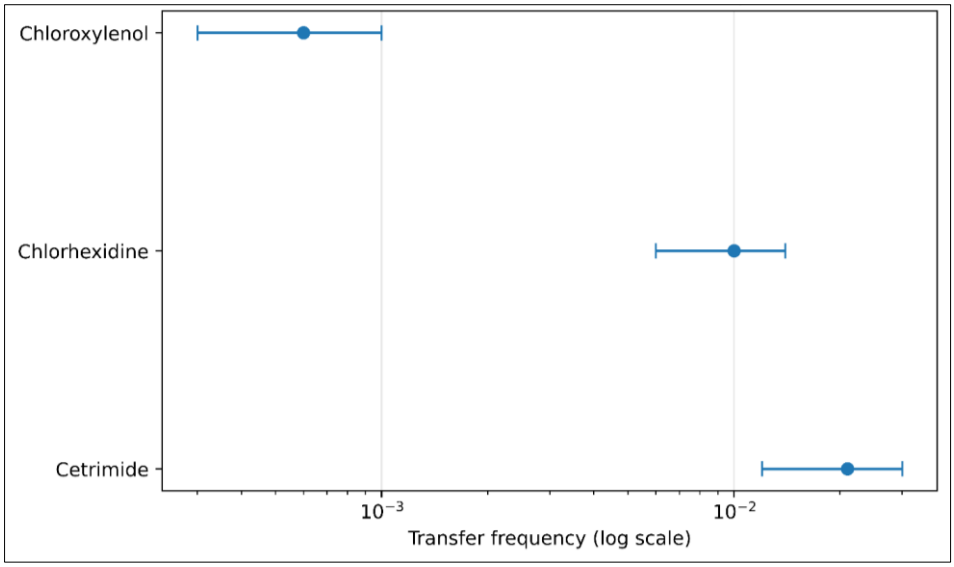

Figure 3: Forest Plot of Mean Transfer Frequencies of Disinfectant Resistance Determinants in Donor and Recipient Escherichia coli Strains (95% Confidence Intervals)

DISCUSSION

Three antibiotics were selected for this experiment and these were tetracycline, trimethoprim and ampicillin and number of resistant serotypes to these antibiotics were selected which were 13, 22 and 9 respectively. The R determinants transfer from clinical isolates of Escherichia coli to *E.coli* K12 and the number were 4, 15 and 7 for tetracycline, trimethoprim and ampicillin and their frequency range of transfer was 3.1 x 10^{-3} -5 x 10^{-2}, 5.2 x 10-4 – 2.1 x 10^{-2} and 5.2 x 10^{-4} – 7.5 x 10^{-3} for tetracycline, trimethoprim and ampicillin respectively. Pearson correlation between donor and recipient transfer frequencies (r) value was 0.396. Moreover, Forty four serotypes were used for metal resistance transfer and only 29, 2 and 6 serotypes showed transfer for lead, zinc and mercury respectively. The frequency range of R resistance determinant transfer for lead, zinc and mercury was 2.6 x 10^{-4} to 8.3 x 10^{-2}, 1 x 10^{-3} to 6.3 x 10^{-3} and 1.8 x10^{-3} to 2.5 x 10^{-2} respectively. The passage of bacteria through diluted disinfectants may not only bring about phenotypic changes in their antibiograms [64], but also changes in phage susceptibility patterns [62, 63]. Sublethal inhibitory concentrations of the disinfectants for different strains did not markedly differ between strains. Passage through disinfectants induced susceptibility to most of the antibiotics in many of the strains in the present study. It was found that, in the case of E. coli, the loss of phosphatases and heptose in the lipopolysaccharide resulted in increased susceptibility to antibiotics, such as novobiocin, spiramycin and actinomycin D [65]. In the present study, a few disinfectants exposed strains became resistant to neomycin and kanamycin, but not to gentamicin or amikacin [1]. The results showed that genes responsible for resistance to Ampicillin, Erythromycin, Chloramphenicol, and Copper may be located on the chromosome**,** while the genes of Tetracycline, Nalidixic acid, and Cobalt may be located on the DNA plasmid. Churchill and Romanus [66], ensured that when they pointed if the resistance of antibiotics or heavy metals was lost after curing that means the resistance was mediated by a plasmid, while if the resistance was not affected, it means chromosomally borne. Zhang *et al.,* [67], pointed out that resistance to antibiotics may remain unaffected after plasmid curing because of the high copy number of the plasmid. *E. coli* has genes that resist ampicillin located on plasmid and chromosomal DNA; Philippon *et al.,* [68], pointed out that *E. coli* have (ESBLs) enzymes (extended–spectrum β–lactamases) that hydrolyze β-Lactam antibiotics, and these enzymes were plasmid–encoded, while Peter *et al.,* [69], showed that *E. coli* has the chromosomal AmpC gene, which is responsible for ampicillin resistance by AmpC β-lactamase. Yang *et al.,* [70], showed that *E. coli* have genes for copper resistance located on the DNA chromosome and the DNA plasmid. Possibly, genes responsible for resistance to cobalt are located on the DNA plasmid; Bhattacherjee *et al.,* [71], pointed out that resistance to heavy metals in bacteria is spread by genes located on the R plasmid in general, and sometimes by mutation and selection. Also, in another study, Bennet [72], pointed out that resistance of antibiotics and heavy metals found on mobile genetic elements: plasmids, transposons, integrons, and gene cassettes, these elements distribute resistance genes among bacteria [37]. Cadmium- and mercury-associated determinants showed the highest mean transfer frequencies, whereas zinc-associated determinants exhibited the lowest transfer efficiencies. Lead resistance determinants demonstrated intermediate transfer rates with considerable variability among isolates. Overall, donor transfer frequencies exceeded recipient transfer frequencies, indicating incomplete establishment of transferred determinants in recipient *E. coli* K12 cells. The problem of hospital cross-infection due to contamination of disinfectants has been recognized [73, 74]. The present study revealed that the present study revealed that the cetrimide transfer frequency from clinical isolates to E, coli K12 and 12 out of 13 serotypes showed resistance transfer with range from 3.4 x 10^{-4} to 4.9 x 10^{-2}; 2 of them were of O125 serotype of donors. It was also noticed that the frequency of resistance transfer for receipt ranged between 1.3 x 10^{-3} to 1.2 x 10^{-1} which was the highest among all antimicrobial agents tested. The forest plot demonstrated variability in the transfer frequencies of disinfectant resistance determinants among *Escherichia coli* isolates. The 95% confidence intervals indicated that cetrimide resistance determinants exhibited the greatest variability in transfer frequency, whereas chloroxylenol resistance determinants showed the narrowest distribution. However, it may be anticipated that disinfectant class influences the role of AMR on disinfectant tolerance. The mode of action of a disinfectant varies depending on its class. For example, aldehydes act by targeting cytoplasmic membrane proteins, causing membrane damage, coagulation of inner cell components and enzyme inhibition [75-77]. However, iodides passively diffuse through the cell membranes, leading to cell death due to oxidation of intracellular components [78, 79]. These modes of action commonly rely on mechanisms that are like those utilized by antibiotics. Consequently, co-resistance between antibiotics and different disinfectant classes has been increasingly observed [46]. However, the current study did not support these findings [46]. Likewise, cross-resistance has been identified between QAC disinfectants and fluoroquinolones [80, 81], due to the influence of multidrug efflux pumps. However, the *C. jejuni* FQR strain used in this study did not show higher tolerance towards QACs. Yet, no difference in susceptibility was observed. These previous studies identified the potential relationship between QACs and fluoroquinolones in a variety of other bacterial species. Therefore, a relationship between QACs and fluoroquinolones may not play the same role in *Campylobacter*. Al-Jebouri [1], found no relationship between antimicrobials and disinfectants in FQR *C. jejuni*. Furthermore, they found high susceptibility to QACs, opposing what was observed in this study.

However, again, they. Bar chart depicting the number of tubes with growth (i.e. tolerance) for each disinfectant class for the sensitive and resistant *E. coli* (pink and purple), *C. jejuni* (light green and dark green), *E. faecium* (orange and red) and *S. aureus* (blue and turquoise) strains, respectively. Total number of tubes per class of disinfectant examined disinfectant susceptibility in standardized broth microdilution assays for 42 h without the inclusion of organic matter. The lack of evidence of cross-resistance between antibiotics and disinfectants may relate to the *Campylobacter* serovar. Different serovars and clonal complexes of *C. jejuni* have been documented to exhibit varying growth capabilities and virulence in a range of environments [81, 82]. Consequently, the chosen strains could be expected to exhibit varying tolerance towards disinfectants due to serovar, irrespective of antibiotic tolerance [83-90].

CONCLUSIONS

Tetracycline resistance determinants exhibited the highest conjugative transfer frequencies, indicating that TE-associated R plasmids are transferred more efficiently than those carrying TMP or AMP resistance. Recipient frequencies were consistently lower than donor frequencies, indicating transfer inefficiency and/or reduced establishment of resistance determinants in the recipient strain (*E. coli* K12). Forty four serotypes were used for metal resistance transfer and only 29, 2 and 6 serotypes showed transfer for lead, zinc and mercury respectively. The present study revealed that the cetrimide transfer frequency from clinical isolates to E, coli K12 and 12 out of 13 serotypes showed resistance transfer with range from 3.4×10^{-4} to 4.9×10^{-2} ; 2 of them were of O125 serotype of donors.

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Statement of Ethics

All the procedures involving human participation were conducted in strict accordance with ethical standards of Institutional Research Committee, Department of Scientific Research, Mosul University as well as the 1964 Helsinki Declaration and its subsequent amendments or equivalent ethical norms.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Conflict of Interest Statement

The author declares that he has no conflicts of interest, financial or otherwise.

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