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Transfer of Antimicrobial-Resistant *Escherichia coli* and Resistance Genes in a Child Care Center

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Running title: Resistance transfer in a child care center

Abstract

Several reports describe antimicrobial-resistance transfer among children and the community in outbreak situations, but transfer between a child and a care giver has not been examined in child care facilities under normal circumstances. We investigated the transfer of antimicrobial-resistance genes, resistant bacteria, or both among healthy children and teachers. From 2007 to 2009, 104 *Escherichia coli* isolates were obtained from four teachers and 38 children in a child care center. Twenty-six cephem-resistant isolates were obtained from children in 2007 and 2008. In 2009, cephem-resistant isolates were detected in children as well as a teacher. Nalidixic acid–resistant isolates from the same teacher for 3 years showed low similarity (<50%) to each other. However, an isolate from a teacher in 2007 and another from a child in 2008 showed high similarity (87%). Pulsed-field gel electrophoresis revealed 100% similarity for four isolates in 2007 and one isolate in 2008, and also similarity among seven isolates carrying the virulence gene (CNF1). This study yielded the following findings: (1) a gene for extended-spectrum β-lactamase was transferred from a child to other children and a teacher; (2) a nalidixic acid–resistant isolate was transferred from a teacher to a child; and (3) a virulent bacterium was transferred between children.

Keywords: *Escherichia coli*, child care center, antimicrobial resistance, transfer

Introduction

More than 80% of urinary tract infections in children are caused by *Escherichia coli* [1], and approximately 8% of children from 1 month to 11 years old experience infections from *E. coli* [2]. As the number of antimicrobial agents to treat *E. coli* urinary tract infection has increased, the occurrence of resistant bacteria has also increased [3]. Antimicrobial-resistant bacteria can cause a serious health risk, especially in children [4]. Children at a child care facility may play a role as a reservoir of resistant bacteria, and they can transfer resistant bacteria to family and community members, resulting in spread of resistance [4-6]. Some reports have described higher detection rates of methicillin-resistant *Staphylococcus aureus* (MRSA) and trimethoprim-resistant *E. coli* in children attending child care facilities than in those who do not attend a child care facility [7-9]. Adcock *et al.* highlighted that resistant strains are spread to families and communities through person-to-person contacts [5]. However, no study has investigated the transfer of antibiotic-resistance genes or antibiotic-resistant bacteria between healthy children and teachers in a child care facility.

Transfer of antimicrobial-resistant bacteria and resistance genes may be a great risk not only to children and teachers, but also to the local community. Therefore, this study aimed to investigate the actual transmission of antibiotic-resistant bacteria and antibiotic-resistant genes in a child care facility.

Materials and Methods

Isolation and identification of E. coli

Fecal samples were obtained from four teachers and 38 children (6 to 36 months old) in a child care center in Gwangmyeong-si, Korea, with sterile cotton swabs and transported to the laboratory in a transport medium. *E. coli* was isolated following the guidelines of Association of Official Analytical Chemists [10]. Samples were inoculated on MacConkey agar plate (BD,

Sparks, MD) by streaking. After incubation for 18–24 h at 35°C, well-isolated pink colonies were obtained and re-inoculated to obtain pure colonies. Each isolate was identified with API 20E (bioMérieux, Marcy l'Étoile, France). Isolates were deposited in the Culture Collection of Antimicrobial Resistant Microbes (CCARM, Seoul, Korea).

Assay of minimal inhibitory concentration

Minimal inhibitory concentrations (MICs) were assayed with the agar dilution method following the guidelines of the Clinical and Laboratory Standards Institute (CLSI) [11]. Tested antimicrobial agents were ampicillin, cephalothin, cefoxitin, cefazolin, cefotaxime, ceftazidime, chloramphenicol, ciprofloxacin, gentamicin, nalidixic acid, norfloxacin, streptomycin, and tetracycline. In case of nalidixic acid–resistant *E. coli*, response to gatifloxacin, gemifloxacin, levofloxacin, and ofloxacin was also tested. Antimicrobial agents were purchased from Sigma (St. Louis, MO) *Escherichia coli* ATCC 25922 was used as a control.

Double disk synergy test

The double-disk synergy test (DDST) was performed with strains resistant to at least one cephalosporin according to the CLSI guideline [11]. Muller-Hinton agar (BD) plates and disks containing 30 μg of ceftazidime or cefotaxime, with and without 10 μg of clavulanic acid, were used for the test. *Klebsiella pneumoniae* ATCC 700603 was used as a control.

Detection of ESBL genes by PCR and sequencing

PCR with specific primers and sequencing were used to characterize the type of β-lactamase. PCR primers and conditions have been described previously [12, 13]. PCR was performed in a total volume of 25 μL, containing 2 μL of template DNA, 10 pmol of specific primers, 1.5

U of *Taq* polymerase, and 200 μM each of dATP, dCTP, dGTP, and dTTP (Biobasic, Ontario, Canada) in a reaction buffer consisting of 100 mM Tris-HCl, 500 mM KCl, and 1 mM $MgCl_2$. PCR was performed using GeneAmp PCR system 9700 (Applied Biosystems, Foster City, CA). After electrophoresis of PCR products on a 1% agarose gel and visualization with ethidium bromide, the products were purified using the QIAquick gel extraction kit (Qiagen, Valencia, CA). DNA sequencing was performed with an ABI 3730XL Sequencer (Applied Biosystems) by Bionics (Seoul, Korea).

Detection of quinolone-resistance mutations

Mutations in quinolone resistance determining regions (QRDRs) of DNA, including gyrase (*gyrA* and *gyrB*) and topoisomerase IV (*parC* and *parE*), were detected by sequencing the genes after PCR with specific primers [14, 15]. PCR amplification of *qnr* and *aac(6')-Ib-cr* was performed as described previously [16-18]. PCR was performed in a total volume of 25 μL, containing 2 μL of template DNA, 10 pmol of specific primer, 1.5 U of *Taq* polymerase, and 200 μM each of dATP, dCTP, dGTP, and dTTP (Biobasic, Ontario, Canada) in a reaction buffer consisting of 100 mM Tris-HCl, 500 mM KCl, and 1 mM $MgCl_2$. PCR was performed using GeneAmp PCR system 9700 (Applied Biosystems). PCR products were treated and sequenced as described for ESBL genes.

Detection of virulence genes by PCR

Multiplex PCR for virulence genes in *E. coli* was performed with primers specific to 11 virulence genes (VE1)-heat-labile (LTI) and heat-stable (STI, STII) toxin; verotoxin types 1, 2, and 2e (VT1, VT2, and VT2e); cytotoxic necrotizing factor (CNF)1 and CNF2; attaching and effacing mechanisms (*eaeA*); enteroaggregative mechanisms (Eagg); and enteroinvasive mechanisms (Einv) as previously described [19]. PCR was performed in a total volume of 25

114 μL, containing 2 μL of template DNA, 10 pmol of specific primer for virulence genes (Table
115 1), 1.5 U of *Taq* polymerase, and 200 μM each of dATP, dCTP, dGTP, and dTTP (Biobasic,
116 Ontario, Canada) in a reaction buffer consisting of 100 mM Tris-HCl, 500 mM KCl, and 1
117 mM $MgCl_2$. PCR was performed using GeneAmp PCR system 9700 (Applied Biosystems),
118 and PCR products were visualized with ethidium bromide after electrophoresis on a 1%
119 agarose gel.
120
121 *Pulsed-field gel electrophoresis*
122 Pulsed-field gel electrophoresis (PFGE) was performed as described in a PulseNet with some
123 modifications [20]. Briefly, bacterial cells were grown overnight on Luria Bertani (LB, BD)
124 agar plates at 37°C. Colonies were suspended in a suspension buffer (100 mM Tris-HCl, 100
125 mM EDTA, pH 8.0), and cell density was adjusted to 1.3–1.4 at 610 nm. The cell suspension
126 (100 μL) was mixed with 5 μL of proteinase K (20 mg/mL) and an equal volume of melted 1%
127 InCert agarose. The mixture was carefully dispensed into a sample mold (Bio-Rad, Hercules,
128 CA). After solidification, plugs were transferred into 2-mL microcentrifuge tubes containing
129 1.5 mL of cell lysis buffer (50 mM Tris-HCl, 50 mM EDTA, 1% sarcosyl, pH 8.0) and 0.5
130 mg/mL of proteinase K. Cells were lysed for 1.5 h in a water bath with continuous agitation.
131 After lysis, plugs were washed twice with sterile distilled water and four times with TE buffer
132 (10 mM Tris-HCl, 1 mM EDTA, pH 8.0) for 15 min per wash at 54°C with gentle shaking.
133 Agarose-embedded DNA was equilibrated in a restriction enzyme buffer for 2 h at 37°C. For
134 complete digestion of DNA, 50 U of *Xba*I (5′-TCTAGA-3′; Takara, Shiga, Japan) was used.
135 Restricted fragments were separated on a 1.0% SeaKem gold agarose gel using CHEF-DR III
136 (Bio-Rad) in 0.5× TBE buffer (44.5 mM Tris, 44.5 mM boric acid, 1 mM EDTA, pH 8.0).
137 Electrophoresis was performed at 6 V/cm for 20 h with pulse time increasing from 2.2 to 54.2
138 s. A lambda DNA ladder, composed of 48.5 kb concatemers (BioLabs, Ipswich, MA), was

used as the size standard. Digital images were stored electronically as TIF files and analyzed with GelCompar software (Applied Maths, Austin, TX).

Results

Isolation and identification of E. coli

Total 104 *E. coli* isolates (14 isolates from four teachers and 90 isolates from 38 children) were obtained from June 2007 to March 2009. Colonies that were produced from the same sample but differed in size or antimicrobial resistance profile were treated as independent isolates.

Minimal inhibitory concentration

Antimicrobial resistance patterns of isolates from children and teachers differed from one another. Isolates from children showed higher resistance rates than those from teachers for ampicillin, cephalothin, cephazolin, cefoxitin, cefotaxim, and streptomycin (Table 2). On the contrary, resistance rates to gentamicin, tetracycline, ciprofloxacin, norfloxaxin, and nalidixic acid were higher in isolates from teachers than those from children (Table 2). MIC_{90}s (minimum inhibitory concentration required to inhibit the growth of 90% of organisms) showed a pattern similar to the resistance rates. MIC_{90}s for cephalothin, cephazolin, cefoxitin, cefotaxim, ceftazidim, and streptomycin in isolates from children were much higher than those of isolates from teachers. MIC_{90}s for gentamicin, norfloxacin, and nalidixic acid were higher in isolates from teachers than in those from children (Table 3).

Two nalidixic acid–resistant *E. coli* isolates were detected in teachers only in 2007. In 2008 and 2009, nalidixic acid–resistant *E. coli* isolates were detected in six children. Cephalothin- and cephazolin-resistant *E. coli* were detected in children in 2007 and 2008 but not in 2009 while they were detected in teachers in 2009 (Table 2). Nalidixic acid–resistant

isolates, CCARM 1F561 from a teacher and CCARM 1F601 and 1F620 from two children showed the same MICs to additional four quinolones- gatifloxacin, gemifloxacin, levofloxacin, and ofloxacin. However, CCARM 1F561 was susceptible to aminoglycosides and tetracycline, but CCARM 1F601 and 1F620 were resistant to aminoglycosides and tetracycline.

Detection of ESBL genes

Eight isolates were identified as ESBL-producing strains by DDST, and all had TEM-type ESBL genes. These isolates included one isolate from a child in 2008 and seven isolates from a teacher and children in 2009.

Quinolone-resistance mechanisms and genotypic similarity of nalidixic acid–resistant E. coli

E. coli ten isolates were resistant to nalidixic acid during the 3-year study period. In 2007, two isolates (CCARM 1F561 and 1F572) from teachers were resistant to nalidixic acid. In 2008 and 2009, nalidixic acid–resistant isolates were found in the same teacher as well as in children. These isolates had mutations previously reported in *gyrA*, *parC*, and *parE* [14, 15, 21-23], but not in *gyrB*. In the case of CCARM 1F572, a new mutation was found at *gyrA* (Pro126 to Leu). CCARM 1F601 and 1F620 had the same MICs and the same mutations in *gryA* and *parC* (Table 4). CCARM 1F561 from a teacher and CCARM 1F601 from a child had the same four mutations (two in *gyrA* and two in *parC*), while CCARM 1F561 from a teacher and 1F620 from a child had the same five mutations (two in *gyrA*, two in *parC*, and one in *parE*). CCARM 1F615 and 1G123 from two children had the same mutations (one in *gyrA* and two in *parE*; Table 4). The *qnr* and *aac(6')-Ib-cr* gene were not detected in nalidixic acid–resistant isolates.

Detection of virulence genes in E. coli

Virulence genes were detected in seven isolates from children, and these were all CNF1. These isolates included six isolates in 2007 and one in 2008.

Pulsed-field gel electrophoresis

High similarity was revealed by PFGE among ESBL-producing isolates, which were all from children (Fig. 1A). CCARM 1G112 showed close similarity (100%) to CCARM 1G125, while CCARM 1G124 showed close similarity (96.3%) to CCARM 1G126. Nalidixic acid–resistant isolates showed close similarity to each other in PFGE (Fig. 1B). CCARM 1F561 from a teacher showed high similarity with CCARM 1F602 (87.5%) and CCARM 1F620 (85.8%) from children. CCARM 1F615 and CCARM 1G123 from children showed very high similarity (92.3%) to each other. In addition, five CNF-producing isolates from children showed 100% similarity to each other (Fig. 1C).

Discussion

Children spend considerable time in day care facilities, where they can be easily exposed to various bacteria, and can be reservoirs for antimicrobial-resistant bacteria amplifying the spread of these bacteria in the community [4-6]. If children carry resistant bacteria, treating infection with antimicrobial agents would be difficult.

In Japan, many children in day care facilities frequently experience acute respiratory infection and otitis media. The percentage of children in day care facilities who carry the four major pathogens (*Streptococcus pneumoniae*, *Hemophilus influenzae*, *Mycobacterium catarrhalis,* and *S. aureus*) is higher than among children who do not attend day care facilities [6]. Adcock (1998) [5] and Carvalho (2017) [9] also reported the possibility of transmission of MRSA to other children in the same facility as well as to their parents. Many studies have

reported the transfer of infectious disease among children or children and teachers. However, no report has provided direct evidence of transfer of antimicrobial-resistance genes or antimicrobial-resistant bacteria, yet.

Results of this study showed different antimicrobial resistance patterns of isolates from children and teachers. For example, isolates from children were more resistant to cephem, while isolates from teachers were more resistant to quinolones. This pattern may be due to the different kinds of antimicrobials prescribed for children and adults. Higher resistance rates were observed in isolates from children for antimicrobial agents (cephem) that are generally prescribed to children. Resistance to nalidixic acid, which is no longer prescribed in the clinical environment, was not observed in children. These results support the previous hypothesis that greater use of antimicrobial agents causes more resistance [24, 25].

Quinolone resistance is mediated by mutation of QRDR region [14, 18, 26], *qnr* gene for plasmid-borne quinolone resistance [17, 26], modification of *aac(6')-Ib-cr* gene [16, 26] , and regulation of gene expression of efflux pumps [14, 26]. In this study, we detected QRDR mutation and genes for resistance mechanisms except efflux mechanism.

Nalidixic acid–resistant isolates were first observed in a teacher in 2007, and then in the same teacher and in children in 2008 and 2009. The isolate from a teacher in 2007 (CCARM 1F561) and the isolate from a child in 2008 (CCARM 1F620) had close similarity (87%) based on PFGE as well as the same point mutations in *gyrA*, *parC*, and *parE.* Moreover, these two isolates had the same MICs to 11 antimicrobials, with the exception of aminoglycosides. CCARM 1F620 had much higher MICs to aminoglycosides than CCARM 1F561. These results suggest that CCARM 1F561 was transferred to a child and then acquired resistance to aminoglycosides. Aminoglycosides are widely prescribed to children for urinary tract infection, septicemia, and skin and bone soft tissue infection (including

ambustion) [27, 28] and serve as first-line treatments for unidentified infection. Although they carry risks for nephrotoxicity and ototoxicity, aminoglycosides are widely prescribed because of their useful antimicrobial activity and low cost. These factors make aminoglycosides as the first choice of antimicrobials for ear infection and bronchitis [29].

Close similarities were observed among ESBL-producing isolates from children and teachers in different years. ESBL-producing isolates were detected only in children in 2008 but in both children and teachers in 2009. Among these, two isolates in 2009 (CCARM 1G112 and 1G125) showed 100% similarity by PFGE and the other two isolates in 2009 (1G124 and 1G126) showed 97% similarity. Except these four isolates, the other isolates showed no similarity. Since all eight ESBL-producing isolates had the same type ESBL-TEM, the gene, not the bacteria, must have been transferred among children and teachers. Results suggested that nalidixic acid–resistant *E. coli* was transferred from teachers to children, while cephalothin and cefazoline-resistant *E. coli* was transferred among children.

We also investigated the transfer of virulent bacteria and virulence genes. Close similarities were observed among seven isolates with the virulence gene CNF1 from children in 2007 and 2008 as well as among quinolone-resistant isolates from a child and a teacher. CNF1 was detected in isolates from children in 2007 as well as 2008. This result showed the transfer of virulent bacteria as well as a virulence gene.

A monitoring system as well as thorough hygiene practices must be performed to prevent the transfer of resistant bacteria from child care providers to children or between children.

Acknowledgements

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349 Table 1. Sequences of PCR primers for virulence gene and product size.

Virulence gene	Primers[a]	Product size (nucleotides)
VT1	fp: 5′-ACGTTACAGCGTGTTGCRGGGATC-3′ bp:5′-TTGCCACAGACTGCGTCAGTRAGG-3′	121
VT2	fp: 5′TGTGGCTGGGTTCGTTAATACGGC-3′ bp:5′-TCCGTTGTCATGGAAACCGTTGTC-3′	102
VT2e	fp: 5′-CCAGAATGTCAGATAACTGGCGAC-3′ bp:5′-GCTGAGCACTTTGTAACAATGGCTG-3′	322
eaeA	fp: 5′-TGAGCGGCTGGCATGAGTCATAC-3′ bp:5′-TCGATCCCCATCGTCACCAGAGG-3′	241
CNF1	fp: 5′-GGCGACAAATGCAGTATTGCTTGG-3′ bp:5′-GACGTTGGTTGCGGTAATTTTGGG-3′	552
CNF2	fp: 5′-GTGAGGCTCAACGAGATTATGCACTG-3′ bp:5′-CCACGCTTCTTCTTCAGTTGTTCCTC-3′	839
LTI	fp: 5′-TGGATTCATCATGCACCACAAGG-3′ bp:5′-CCATTTCTCTTTTGCCTGCCATC-3′	360
STI	fp: 5′-TTTCCCCTCTTTTAGTCAGTCAACTG-3′ bp:5′-GGCAGGATTACAACAAAGTTCACAG-3′	160
STII	fp: 5′-CCCCCTCTCTTTTGCACTTCTTTCC-3′ bp:5′-TGCTCCAGCAGTACCATCTCTAACCC-3′	423
Einv	fp: 5′-TGGAAAAACTCAGTGCCTCTGCGG-3′ bp:5′-TTCTGATGCCTGATGGACCAGGAG-3′	140
Eagg	fp: 5′-AGACTCTGGCGAAAGACTGTATC-3′ bp:5′-ATGGCTGTCTGTAATAGATGAGAAC-3′	194

350 [a] fp, forward primer; bp, backward primer; R = A and G.

Table 2. Resistance rate of *E. coli* isolated from a child care center.

Class and antimicrobial agent	*2007*		*2008*		*2009*		*Total*		
	Teacher (n = 10)[a]	*Child (n = 44)*	*Teacher (n = 2)*	*Child (n = 21)*	*Teacher (n = 2)*	*Child (n = 25)*	*Teacher (n = 14)*	*Child (n = 90)*	*Total (n = 104)*
Penicillin									
Ampicillin	4 (40.0)[b]	23 (52.3)	1 (50.0)	13 (61.9)	1 (50.0)	15 (60.0)	6 (42.9)	51 (56.7)	57 (54.8)
Cephem									
Cephalothin	0 (0.0)	10 (22.7)	0 (0.0)	6 (28.6)	1 (50.0)	10 (40.0)	1 (7.1)	26 (28.9)	27 (26.0)
Cefazoline	0 (0.0)	5 (11.4)	0 (0.0)	2 (9.5)	1 (50.0)	6 (24.0)	1 (7.1)	13 (14.4)	14 (13.5)
Cefoxitin	0 (0.0)	2 (4.5)	0 (0.0)	3 (14.3)	0 (0.0)	0 (0.0)	0 (0.0)	5 (5.6)	5 (4.8)
Cefotaxime	0 (0.0)	0 (0.0)	0 (0.0)	1 (4.8)	0 (0.0)	4 (16.0)	0 (0.0)	5 (5.6)	5 (4.8)
Ceftazidime	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Aminoglycoside									
Gentamicin	0 (0.0)	0 (0.0)	1 (50.0)	3 (14.3)	1 (50.0)	1 (4.0)	2 (14.3)	4 (4.4)	6 (5.8)
Streptomycin	1 (10.0)	5 (11.4)	0 (0.0)	2 (9.5)	0 (0.0)	6 (24.0)	1 (7.1)	13 (14.4)	14 (13.5)
Tetracycline									
Tetracycline	3 (30.0)	9 (20.5)	1 (50.0)	8 (38.1)	1 (50.0)	10 (40.0)	5 (35.7)	27 (30.3)	32 (30.8)
Fluoroquinolone									
Ciprofloxacin	1 (10.0)	0 (0.0)	0 (0.0)	2 (9.5)	0 (0.0)	0 (0.0)	1 (7.1)	2 (2.2)	3 (2.9)
Norfloxacin	1 (10.0)	0 (0.0)	0 (0.0)	2 (9.5)	0 (0.0)	0 (0.0)	1 (7.1)	2 (2.2)	3 (2.9)
Quinolone									
Nalidixic acid	2 (20.0)	0 (0.0)	1 (50.0)	5 (23.8)	1 (50.0)	1 (4.0)	4 (28.6)	6 (6.7)	10 (9.6)
Phenicol									
Chloramphenicol	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)

[a]Number of *E. coli* isolates.
[b]Values in parentheses are percentages.

Table 3. MIC_{50} and MIC_{90} of *E. coli* isolated from a child care center.

Class and antimicrobial agent	*Teachers (n=14)*		*Children (n=90)*	
	MIC_{50}	*MIC_{90}*	*MIC_{50}*	*MIC_{90}*
Penicillin				
Ampicillin	8	>128	32	>128
Cephems				
Cephalothin	8	16	8	>128
Cefazoline	2	8	4	>128
Cefoxitin	4	4	4	8
Cefotaxime	≤0.5	≤0.5	≤0.5	1
Ceftazidime	≤0.5	≤0.5	≤0.5	1
Aminoglycosides				
Gentamicin	1	64	1	1
Streptomycin	8	16	4	128
Tetracyclines				
Tetracycline	1	128	1	128
Fluoroquinolones				
Ciprofloxacin	≤0.5	≤0.5	≤0.5	≤0.5
Norfloxacin	≤0.5	1	≤0.5	≤0.5
Quinolone				
Nalidixic acid	4	128	4	4
Phenicols				
Chloramphenicol	8	8	8	8

MIC_{50}, 50% minimum inhibitory concentration; MIC_{90}, 90% minimum inhibitory concentration.

Table 4. Mutations detected in the DNA topoisomerase genes of *E. coli*

Strain no.	Year		*gyrA*		*parC*		*parE*	
			Amino acid	Substitution	Amino acid	Substitution	Amino acid	Substitution
1F561	2007	Teacher-HSE	Ser83	Leu	Ser80	Ile	Ile529	Leu
			Asp87	Asn	Glu84	Val		
1F572	2007	Teacher-LSH	Ser83	Leu				
			Pro126	**Leu**				
1F601	2008	Child-PHJ	Ser83	Leu	Ser80	Ile		
			Asp87	Asn	Glu84	Val		
1F613	2008	Teacher-HSE	Ser83	Leu			Ile529	Leu
1F615	2008	Child-KSJ	Ser83	Leu			Ser458	Ala
							Ile529	Leu
1F619	2008	Child-YCM	Ser83	Leu				
1F620	2008	Child-KDH	Ser83	Leu	Ser80	Ile	Ile529	Leu
			Asp87	Asn	Glu84	Val		
1F628	2008	Child-WWB	Ser83	Leu				
1G123	2009	Child-YCS	Ser83	Leu			Ser458	Ala
							Ile529	Leu
1G130	2009	Teacher-HSE	Ser83	Leu				

Bold, new mutation in *gyrA*

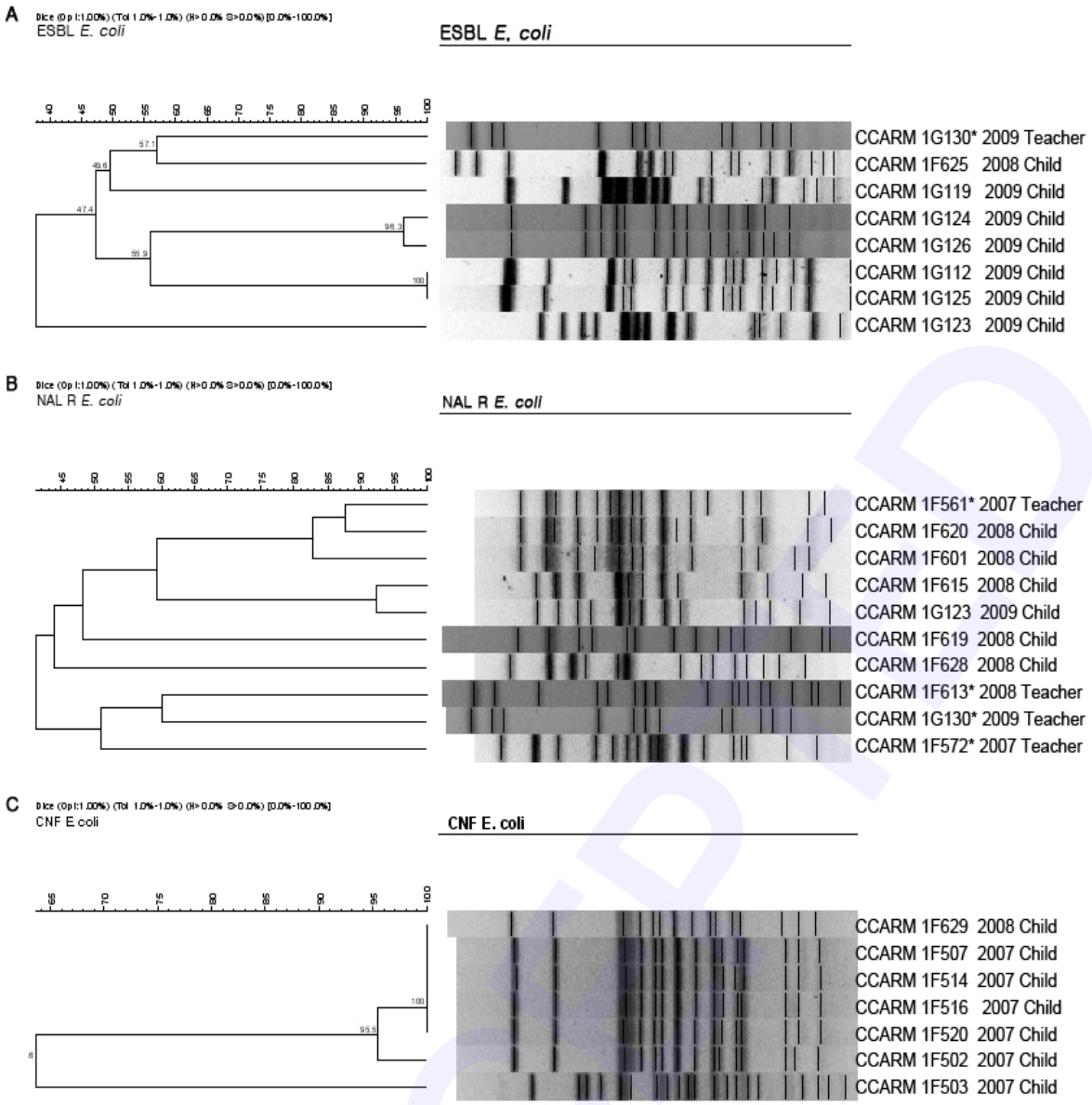

Fig. 1. Figure 1. Dendrogram of E. coli isolates based on PFGE profiles of XbaI-digested genomic DNA using DNA Gel-compar software. A, ESBL-producing isolates; B, nalidixic acid-resistant isolates; C, CNF-producing isolates