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Conjugative Transfer of Plasmid Encoding Extended-Spectrum Beta-Lactamase to Recipient *Salmonella* Strains

Koniugacyjny transfer plazmidu kodującego geny odpowiedzialne za syntezę β-laktamaz o rozszerzonym spektrum substratowym do komórek biorców *Salmonella* sp.

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Abstract

Background. *Salmonella enterica* subsp. *enterica* is one of the most frequently occurring etiological factors of severe gastroenteritis in children. Young children are also commonly colonized by bacterial strains producing ESBL enzymes. These enzymes are encoded on plasmids and can be easy transferred by conjugation to other Gram-negative strains, especially from fecal flora.
Objectives. This study examined the transferability of ESBL plasmid from *Klebsiella pneumoniae* strains to clinical *Salmonella* isolates and then between clinical *Salmonella* strains, which leads to *Salmonella* resistance to beta-lactam antibiotics, the most often used in treatment.
Material and Methods. The transferability of β-lactam resistance determinants was studied by the bacterial conjugation method. *K. pneumoniae* ATCC 700603 carrying SHV-18 ESBL genes and a clinical isolate of *K. pneumoniae* 20 (ESBL$^+$) were used as the donors of ESBL plasmid. *S. enterica* serovar Enteritidis (n = 10), *S. enterica* serovar Typhimurium (n = 10), and *S. enterica* serovar Hadar (n = 10) isolates were used as the recipient strains. Before and after conjugation the MICs of amikacin (AK), ampicillin (AM), ceftriaxone (TX), ceftazidime (TZ), trimethoprim-sulfametoksazol (TS), and ciprofloxacin (CI) were measured by the E-test. All isolates were tested for ESBL activity by the disc diffusion test (the Jarlier Double-Disc test) and the E-test. The plasmid DNA patterns were analyzed by the alkaline method.
Results. All *S.* Enteritidis (10/10), several *S.* Typhimurium (4/10), and one *S.* Hadar (1/10) isolate became transconjugants. All *Salmonella* transconjugants were identified as ESBL producers. After conjugation, increases in the MICs of AM, TX, and TZ were observed.
Conclusion. The efficiency of ESBL plasmid transferability depended on the *Salmonella* serovar. The *S. enterica* serovar Enteritidis strains were the best ESBL plasmid recipients among the clinical *Salmonella* serovars (**Adv Clin Exp Med 2009, 18, 1, 63–70**).

Key words: *Salmonella*, horizontal genes transfer, ESBL.

Streszczenie

Wprowadzenie. Pałeczki *Salmonella enterica* subsp. *enterica* są jednym z najczęstszych czynników etiologicznych *gastroenteritis* u dzieci. Przewód pokarmowy dzieci jest często kolonizowany przez szczepy bakteryjne wytwarzające enzymy typu ESBL, których genetyczne determinanty zlokalizowane w plazmidach mogą być łatwo przekazywane na drodze koniugacji, szczególnie w obrębie bakterii Gram-ujemnych.
Cel pracy. Określenie częstości koniugacyjnego transferu plazmidów typu ESBL ze szczepów *Klebsiella pneumoniae* do komórek szczepów należących do przedstawicieli trzech serowarów *Salmonella*: Enteritidis, Typhimurium i Hadar oraz w obrębie pałeczek *Salmonella*.

Materiał i metody. Częstość przekazywania plazmidów typu ESBL określono metodą koniugacji w podłożu bulionowym. Jako dawców plazmidu typu ESBL zastosowano szczepy *K. pneumoniae* ATCC 700603 (SHV-18) i *K. pneumoniae* 20 (ESBL+). Szczepy *S. enterica* serowar Enteritidis (n = 10), *S. enterica* serowar Typhimurium (n = 10) i *S. enterica* serowar Hadar (n = 10) wykorzystano jako biorców. Wartości MIC dla amikacyny (AK), ampicyliny (AM), ceftriaksonu (TX), ceftazydymu (TZ), trimetoprimu z sulfametoksazolem (TS) i ciprofloksacyny (CI) określono metodą E-testów przed i po koniugacji. Zdolność do syntezy enzymów ESBL badano metodą dwóch krążków (DDST) wg Jarliera oraz metodą E-testów. Analizę profili plazmidowego DNA przeprowadzono metodą lizy alkalicznej.
Wyniki. W przypadku wszystkich badanych szczepów *S.* Enteritidis (10/10), czterech *S*. Typhimurium (4/10) i jednego izolatu *S*. Hadar (1/10) uzyskano transkoniuganty, które wykazywały produkcję beta-laktamaz o rozszerzonym spektrum substratowym (ESBL). Zaobserwowano wzrost wartości MIC dla AM, TX i TZ wobec uzyskanych transkoniugantów.
Wniosek. Wyniki badań wskazują, że skuteczność i częstość koniugacyjnego transferu plazmidu typu ESBL była różna dla poszczególnych serowarów *Salmonella*. Szczepy *S*. Enteritidis charakteryzowały się największą zdolnością do uzyskiwania plazmidu ESBL (**Adv Clin Exp Med 2009, 18, 1, 63–70**).

Słowa kluczowe: *Salmonella*, horyzontalny transfer genów, ESBL.

Bacteria of *Salmonella* strains are among the most commonly isolated etiological factors of digestive tract infections in humans. The *S.* Enteritidis serovar has been the predominant causative agent in Poland over the past decade, being responsible for over 95% of cases. Other commonly diagnosed serological types include *S.* Typhimurium and *S.* Hadar. This is evidenced in the annual reports of the State Hygiene Department [1, 2]. As shown in the data of the European Commission from 25 membership countries presented in the report "EFSA's First Community Summary Report on Trends and Sources of Zoonoses, Zoonotic Agents and Antimicrobial Resistance in the European Union in 2004", of 380,000 cases of infection of animal origin, more than half were due to *Salmonella* infection [3].

Apart from salmonellosis of typical location, extraintestinal infections caused by *Salmonella* are becoming increasingly common [4–6]. These infections are generally characterized by a severe course, and the majority of the risk-group patients (children below the age of 4 and people over 60) require adequate antibacterial therapy as well as hospitalization [7]. From several dozen to over 100 cases of extraintestinal *Salmonella* infections are confirmed bacteriologically in Poland every year [1, 2].

Increasing resistance of *Salmonella* species isolated from humans to antibiotics and chemotherapeutics has been observed all over the world. The appearance of *Salmonella* extended-spectrum beta-lactamase (ESBL)-positive species raises a special concern as it precludes the administration of the majority of beta-lactam antibiotics, including third-generation cephalosporins, which have been used as the drugs of choice in the therapy of extraintestinal salmonellosis, especially in children [8]. *Salmonella* bacteria which produce ESBL have been isolated all over the world, both from sporadic cases in humans and from epidemic foci. The resistance of *Salmonella* to beta-lactam antibiotics is always of a secondary character, the conditioning genes being located outside the bacterial chromosome. This mechanism of *Salmonella* resistance is very dangerous from the epidemiological point of view as it enables the horizontal transfer of genes determining resistance, which are located on conjugational plasmids, transposons, or integron cassettes in the differentiated and multi-species intestinal flora [9–11]. The increasing drug resistance of these pathogens is especially worth consideration in view of the high virulence of *Salmonella* species and the fact that they more commonly occur outside the hospital.

The aim of the study was to determine the frequency of conjugational transfer of ESBL plasmids from *K. pneumoniae* to strains belonging to three *Salmonella* serovars: Enteritidis, Typhimurium, and Hadar, as well as between *Salmonella* bacilli.

Material and Methods

Bacterial Strains

Thirty *S. enterica* clinical isolates belonging to the Enteritidis (n = 10), Typhimurium (n = 10), and Hadar (n = 10) serovars from human fecal samples were collected. The bacterial strains were isolated from 2002 to 2005 in a variety of centers, i.e. the Bacteriological Laboratory, J. Korczak Lower Silesian Pediatric Center, Wrocław (10 strains of *S*. Enteritidis), the County Sanitary-Epidemiological Station, Wrocław (5 strains of *S*. Typhimurium and 5 of *S*. Hadar), the Polish Collection of Microorganisms, Polish Academy of Sciences, Institute of Immunology and Experimental Therapy, Wrocław (5 strains of *S*. Typhi-

murium), and the Microbial Collection of the Department of Molecular Microbiology and Serology National Salmonella Center, Gdynia (5 strains of *S*. Hadar), Poland, and confirmed by the State Sanitary-Epidemiological Agency. The strain *K. pneumoniae* ATCC 700 603, carrying SHV-18 ESBL genes [12], and a clinical isolate of *K. pneumoniae* ($ESBL^+$) were used as the donors of ESBL plasmid. The *E. coli* $K_{12}C_{600}$ strain lacking plasmid DNA was used as the control strain in the conjugation experiments.

Antibiotic Susceptibility

The susceptibility to selected antimicrobials was tested by the E-test (AB Biodisk) method according to Clinical Laboratory Standards Institute (CLSI) guidelines [13]. The minimal inhibitory concentrations (MICs) of amikacin (AK), ampicillin (AM), ciprofloxacin (CI), trimethoprim-sulfametoksazole (ST), cefotaxime (TX), and ceftazidime (TZ) were estimated. *E. coli* strain ATCC 25922 was used as the quality reference strain.

ESBL Test

According to the guidelines of the CLSI [13] and the Polish Reference Center for Microorganism Drug Sensitivity, extended-spectrum beta-lactamases (ESBLs) were determined by the double-disk synergy test (DDST) of Jarlier et al. [14]. Additionally, the ability of the donor strains to produce ESBLs was confirmed and the obtained transconjugants were determined by measuring the MICs for cefepim (PM) and cefepim with clavulanic acid (PML) on an ESBL gradient strip (AB Biodisk).

Conjugation Experiment

The transmission of plasmids coding for ESBLs was done by conjugation on broth medium. The experiments were performed using *Salmonella* acceptor strains (recipients) which were nalidixic acid sensitive (Na^S) or resistant (Na^R) by two methods: 1) when the donor strain was sensitive to nalidixic acid and resistant to ceftazidime (Na^S, TZ^R, $ESBL^+$) and the recipient strain was resistant to nalidixic acid and sensitive to ceftazimide (Na^R, TZ^S, $ESBL^-$) and 2) when the donor strain (Na^S, TZ^R, $ESBL^+$) and recipient strain were sensitive to nalidixic acid (Na^S, TZ^S, $ESBL^-$). Broth cultures of the donor and recipient strains (logarithmic phase) were mixed at a ratio of 1:1 and incubated at 37°C for 24 h. Transconjugants were selected on SS-agar supplemented with 64 µg/ml nalidixic acid and 4 µg/ml ceftazidime (when the recipient was resistant to nalidixic acid) or on selective medium SS-agar supplemented with 4 µg/ml ceftazidime (when the recipient was sensitive to nalidixic acid). The transfer rate of ESBL-encoding plasmids was calculated from the ratio of the transconjugant's cfu (colony forming unit) to the donor's cfu. Isolated transconjugants were identified biochemically and serologically. Donor cross-check with the recipient *E. coli* $K_{12}C_{600}$ deprived of plasmid DNA was performed as a control.

An abbreviated record of the transconjugant was introduced for the purpose of the study, for example 165(20) and 165(700 603). The first figure (165) denotes the number of the recipient strain (*S.* Enteritidis No. 165), while the figures in brackets (20, 700 603) denote the number of the plasmid donor strain (*K. pneumoniae* 20, *K. pneumoniae* 700 603).

Plasmid DNA Preparation

Plasmid DNA was extracted from the donor strains and their transconjugants by the alkaline method using the Plasmid Mini AX (A&A Biotechnology) according to the manufacturer's procedure. The recipient strains were incubated in LB broth (30 ml) and the donor and transconjugant strains were incubated in LB broth (30 ml) supplemented with ceftazidime (4 µg/ml) at 37°C for 18 h with shaking. Plasmids were electrophoresed by the method of Kado and Liu [15]. The *E. coli* V517 strain was used as a standard.

Results and Discussion

Transmission of ESBL Plasmids

The aim of the study was to compare the effectiveness and prevalence of conjugational transfer of ESBL plasmids to cells of *Salmonella* ESBL recipient species belonging to three serovars which are most commonly isolated in Poland: *S.* Enteritidis, *S.* Typhimurium, and *S.* Hadar [16]. The findings are presented in Table 1. Using the reference strain *K. pneumoniae* 700 603 as the donor of ESBL plasmid for the 10 clinical recipients of *S.* Enteritidis ESBL-negative strains from children treated at the J. Korczak Lower Silesian Pediatric Center in Wrocław, transconjugants were obtained in all the crosses. Of these recipient strains, the most prevalent were those resistant to nalidixic acid. In the *K. pneumoniae* 20 donor, transconjugants were obtained with 8 species of

S. Enteritidis recipients (Table 1). The transfer frequencies of the plasmids responsible for ESBL synthesis in the *S.* Enteritidis recipients ranged from 10^{-6} to 10^{-3}.

Among the 10 investigated *S.* Typhimurium strains, the most prevalent were those sensitive to nalidixic acid. Only two of them, Nos. 244 and 389, were resistant to this antibiotic. *S.* Typhimurium appeared to be a weak recipient of ESBL-encoding plasmids compared with *S.* Enteritidis. When the reference strain *K. pneumoniae* 700 603 was used as the plasmid donor and the 10 strains of *S.* Typhimurium were the recipients, transconjugants were obtained only in two recipient strains, while when using the *K. pneumoniae* 20 donor there were four effective crosses (Table 1).

All the investigated *S.* Hadar strains, regardless of their origin, were resistant to nalidixic acid. The *S.* Hadar strains were practically incapable of accepting plasmid-encoding genes responsible for ESBL synthesis from the donors. The only exception was the cross between the donor *K. pneumoniae* 20 ESBL$^+$ and the recipient *S.* Hadar 55 strain, which produced transconjugants at the very low frequency of 10^{-8}. Although *Salmonella* strains belonging to this serological type reveal many virulence traits, they are characterized by a lack of the virulence plasmid typical of the Enteritidis and Typhimurium serovars. It seems probable that the chromosomal location of the traits of increased virulence in *S.* Hadar strains results from evolution and a yet unexplained mechanism blocks the acquisition of plasmid DNA by conjugation, including ESBL-type plasmids. This hypothesis may be confirmed by the fact that although the strains belonging to the Hadar serovar are the third most prevalent in human infections, the *Salmonella* ESBL$^+$ strain belonging to this serological type has not been isolated in Poland so far [17].

All the obtained *Salmonella* ESBL$^+$ transconjugants belonging to the Enteritidis and Typhimurium serovars which were used as donors in the studies on plasmid transfer in *Salmonella* species were capable of effective conjugation with the *E. coli* $K_{12}C_{600}$ recipient deprived of plasmid DNA with frequencies ranging from 10^{-5} to 10^{-4}. Szych et al. [18] obtained a broader range of conjugation frequency. The data presented in the present study also involved the transfer of plasmids from ESBL$^+$ *Salmonella* strains to the cells of the non-plasmid *E. coli* $K_{12}C_{600}$ strain. The transfer frequencies ranged from 10^{-5} to 10^{-1}. These significant differences were probably due to the fact that Szych et al. [18] investigated clinical *Salmonella* species belonging to six serological types. Dimitrov et al. [19], who investigated the donor properties of a clinical *S.* Typhimurium ESBL$^+$ strain, demonstrated that it was capable of conjugational transfer of this trait to recipient *E. coli* cells with a frequency of 1.2×10^{-6}. Studies performed by Franiczek et al. [20] on other

Table 1. Transconjugants of *S.* Enteritidis and *S.* Typhimurium strains obtained by conjugation with two *K. pneumoniae* donor strains

Tabela 1. Transkoniuganty *S.* Enteritidis i *S.* Typhimurium uzyskane metodą koniugacji z dwoma szczepami dawców *K. pneumoniae*

Recipient strains (Szczepy biorcy)	Transconjugants with *K. pneumoniae* 700 603 donor strain (Transkoniuganty uzyskane z dawcą *K. pneumoniae* 700 603)	Transconjugants with *K. pneumoniae* 20 donor strain (Transkoniuganty uzyskane z dawcą *K. pneumoniae* 20)
S. Enteritidis 165	165	165
S. Enteritidis 447	447	447
S. Enteritidis 518	518	518
S. Enteritidis 634	634	nt
S. Enteritidis 684	684	684
S. Enteritidis 685	685	nt
S. Enteritidis 688	688	688
S. Enteritidis 730	730	730
S. Enteritidis 1104	1104	1104
S. Enteritidis 1217	1217	1217
S. Typhimurium 244	244	244
S. Typhimurium 389	389	389
S. Typhimurium 1035	nt	1035
S. Typhimurium 1048	nt	1048
S. Typhimurium 300, 301, 547, 1004, 1009, 1051	nt	nt

nt – no transconjugant obtained.
nt – nie uzyskano transkoniugantów.

Table 2. Transfer of ESBL plasmids between *Salmonella* strains

Tabela 2. Transfer plazmidów ESBL między szczepami *Salmonella*

S. Enteritidis donor strains (Szczepy dawców *S*. Enteritidis)	Number of transconjugants obtained with the recipients (Liczba transkoniugantów uzyskanych z biorcami)		
	S. Enteritidis n = 8	*S*. Typhimurium n = 2	*S*. Hadar n = 10
165 (700 603)	6/8	2/2	0/10
1217 (700603)	8/8	2/2	0/10
165 (20)	7/8	2/2	0/10
1217 (20)	7/8	2/2	0/10

Enterobacteriaceae strains producing enzymes of ESBL type demonstrated that the transfer frequencies of encoding plasmids to the recipient *E. coli* $K_{12}C_{600}$ strain were from 10^{-5} to 10^{-1}.

The next stage of the present investigations involved the transfer of ESBL-type plasmids in the *Salmonella* species. The novelty of this study consisted of the use of *Salmonella* species isolated from human infections as recipients of plasmid DNA; thus the results could not be compared with similar findings by other authors. Data from the literature indicate that $ESBL^+$ transconjugants were most commonly obtained only to confirm the plasmid location of the determinants of this type of bacterial resistance to beta-lactam antibiotics with the use of non-plasmid mutants of various strains, most commonly *E. coli*, as recipients [4, 21].

To cross *Salmonella* spp., *Salmonella* transconjugants obtained from heterogenic crosses, i.e. *K. pneumoniae* 700 603 × *Salmonella* spp. and *K. pneumoniae* 20 × *Salmonella* spp., which were characterized by the plasmid-conditioned ability to produce ESBL-type enzymes, were used as donors. The transconjugants were resistant to ceftazidim and sensitive to nalidixic acid (Na^S, TZ^R, $ESBL^+$). The recipients of the ESBL plasmids were *Salmonella* resistant to nalidixic acid and sensitive to ceftazidim (Na^R, TZ^S, $ESBL^-$). Of the 20 *Salmonella* strains which fulfilled these criteria, 8 belonged to Enteritidis, 10 to Hadar, and 2 to Typhimurium serovar. The results of conjugation in *Salmonella* spp. are presented in Table 2. It is worth mentioning that transconjugants of *S*. Hadar with *Salmonella* donors were not obtained. The findings suggest that the effectiveness and frequency of conjugational plasmid transfer responsible for the synthesis of ESBL-type enzymes from *K. pneumoniae* to *Salmonella* cells and between *Salmonella* species varied depending on the donors and recipients used in the experiment. *Salmonella* species belonging to the most commonly isolated serovar, Enteritidis, acquired additional plasmids in the conjugation process more easily than the investigated species of *S*. Typhimurium and *S*. Hadar. Unrestricted transfer alone may constitute a hazard of widespread distribution of a genetic determinant of antibiotic resistance within other species and the kinds of bacteria constituting intestinal flora. Studies performed on three groups of neonates in a Turkish hospital demonstrated that the participation of $ESBL^+$ strains in the neonates' intestinal flora was similar and ranged from 26 to 40% [9].

As shown in the present study, the frequency of conjugational transfer of ESBL-type plasmids between the *Salmonella* species varied and ranged from 10^{-8} to 10^{-3}. Comparison of the acceptor properties of the investigated *Salmonella* strains revealed that transconjugants were obtained less frequently in homogenous (*Salmonella* × *Salmonella*) than in heterogeneous crosses (*Klebsiella* × *Salmonella*). These results are consistent with the observations of Franiczek et al. [20] on a large group of *Enterobacteriaceae* strains other than *Salmonella*.

Plasmid Fingerprinting Analysis

The electrophoretic distribution of plasmid DNA provided an additional confirmation of ESBL-type plasmid transfer from donor to recipient cells. A strain deprived of plasmid DNA, *E. coli* $K_{12}C_{600}$, and its transconjugant with *K. pneumoniae* 20, *E. coli* $K_{12}C_{600}$ (20), were used as controls (Fig. 1). Comparison of the plasmid profiles of the donor and recipient strains and their $ESBL^+$ transconjugants confirmed the presence of strands characteristic of donors in transconjugant cells.

The main aim of the study devoted to the analysis of plasmid profiles of primary *Salmonella* strains and their transconjugants was to produce indirect evidence that genes encoding ESBLs were transferred to recipient cells in the plasmids. The results confirmed the presence of additional plasmids in the transconjugant cells, with their size corresponding to plasmid DNA, the presence of which was also found in the cells of the applied

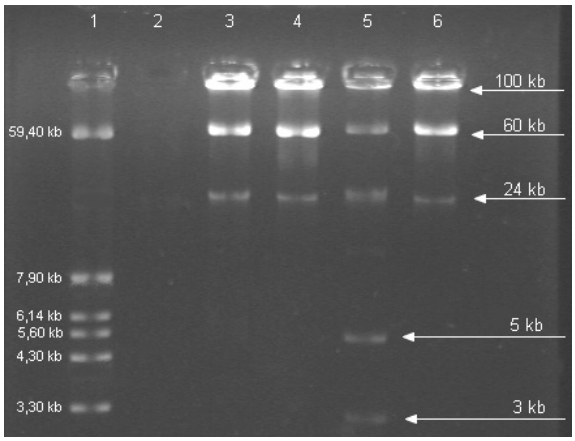

Fig. 1. Plasmid fingerprinting analysis. Lanes: 1, *E. coli* V517; 2, *E. coli* $K_{12}C_{600}$ – recipient; 3, *S.* Enteritidis 518(20) – transconjugant; 4, *E. coli* $K_{12}C_{600}$518(20) – transconjugant; 5, *K. pneumoniae* 20 – donor; 6, *E. coli* $K_{12}C_{600}$(20) – transconjugant

Ryc. 1. Analiza plazmidowego DNA. Ścieżki: 1, *E. coli* V517; 2, *E. coli* $K_{12}C_{600}$ – biorca; 3, *S.* Enteritidis 518(20) – transkoniugant; 4, *E. coli* $K_{12}C_{600}$518(20) – transkoniugant; 5, *K. pneumoniae* 20 – dawca; 6, *E. coli* $K_{12}C_{600}$(20) – transkoniugant

donors capable of transferring ESBL plasmids. A detailed analysis of the distribution of plasmid DNA demonstrated the presence of large virulence plasmids occurring naturally in some serovars of *S. enterica* subspecies. In the case of *S*. Enteritidis this was ~60 kb and in *S*. Typhimurium ~100 kb, while plasmid DNA was not found in cells of *S*. Hadar strain. *S*. Hadar strains isolated from humans, often described as one of the most invasive *Salmonella* strains, do not contain the virulence plasmid [22, 23]. A 2006 report which described 17 *S*. Hadar isolates obtained from the bison is an exception [24]. Genetic examination of these strains demonstrated the presence of plasmids as large as 70 kb.

Antibiotic Susceptibility

The data presented in Table 3 demonstrate the range of MIC values as well as the susceptibilities of all the primary *Salmonella* ESBL-negative strains and ESBL-positive transconjugants to antibiotics. Similarly to the recipient *Salmonella*, they were found to be fully sensitive to AK, CI, and TS. All *Salmonella* transconjugants were characterized by resistance to AM and resistance or reduced sensitivity to TZ and TX and they were positive in ESBL tests (DDST and E-test ESBL).

An increasing participation of multiresistant *Salmonella* species in human infections has been recently observed all over the world [25]. The resistance of *Salmonella* species isolated in Poland to antibacterial agents has also been found to be on the increase [26]. Especially strains belonging to the *S.* Hadar or *S*. Tyhimurium serovars reveal multiresistance to antibacterial agents [8, 23, 27, 28]. This is caused, for example, by the use of fluorochinolones and third-generation cephalosporins in the treatment of acute salmonellosis, which results in the development of strains with reduced susceptibility to ciprofloxacin and ESBL$^+$ strains. Studies on drug sensitivity demonstrated that all

Table 3. MIC (μg/ml) values of antibiotics for donor, recipient, and transconjugant strains

Tabela 3. Wartości MIC (μg/mL) antybiotyków wobec szczepów dawców, biorców i transkoniugantów

Antibiotic (Antybiotyk)	Donors (Dawcy)		Recipients (Biorcy)	Transconjugants (Transkoniuganty)		Recipients (Biorcy)	Transconjugants (Transkoniuganty)		Recipients (Biorcy)	Transconjugants (Transkoniuganty)
	K. pneumoniae 700 603 n = 1	*K. pneumoniae* 20 n = 1	*S.* Enteritidis n = 10	*S.* Enteritidis (700 603) n = 10	*S.* Enteritidis (20) n = 8	*S.* Typhimurium n = 10	*S.* Typhimurium (700 603) n = 2	*S.* Typhimurium (20) n = 4	*S.* Hadar n = 10	*S.* Hadar (20) n = 1
AK	$\leq 16^S$	$\leq 16^S$	$\leq 16^S$ (10)	$\leq 16^S$ (10)	$\leq 16^S$ (8)	$\leq 16^S$ (10)	$\leq 16^S$ (2)	$\leq 16^S$ (4)	$\leq 16^S$ (10)	$\leq 16^S$ (1)
AM	$\geq 32^R$	$\geq 32^R$	$\leq 8^S$ (10)	$\geq 32^R$ (10)	$\geq 32^R$ (8)	$\leq 8^S$ (7) $\geq 32^R$ (3)	$\geq 32^R$ (2)	$\geq 32^R$ (4)	$\leq 8^S$ (4) $\geq 32^R$ (6)	$\geq 32^R$ (1)
CI	$\leq 1^S$	$\leq 1^S$	$\leq 1^S$ (10)	$\leq 1^S$ (10)	$\leq 1^S$ (8)	$\leq 1^S$ (10)	$\leq 1^S$ (2)	$\leq 1^S$ (4)	$\leq 1^S$ (10)	$\leq 1^S$ (1)
TS	$\leq 2^S$	$\leq 2^S$	$\leq 2^S$ (10)	$\leq 2^S$ (10)	$\leq 2^S$ (8)	$\leq 2^S$ (10)	$\leq 2^S$ (2)	$\leq 2^S$ (4)	$\leq 2^S$ (10)	$\leq 2^S$ (1)
TX	$16–32^I$	$16–32^I$	$\leq 8^S$ (10)	$16–32^I$ (8) $\geq 64^R$ (2)	$16–32^I$ (6) $\geq 64^R$ (2)	$\leq 8^S$ (10)	$16–32^I$ (2)	$16–32^I$ (1) $\geq 64^R$ (3)	$\leq 8^S$ (10)	$16–32^I$ (1)
TZ	$\geq 32^R$	$\geq 32^R$	$\leq 8^S$ (10)	16^I (7) $\geq 32^R$ (3)	$\geq 32^R$ (8)	$\leq 8^S$ (10)	16^I (2)	$\geq 32^R$ (4)	$\leq 8^S$ (10)	$\geq 32^R$ (1)

S – sensitivity.
R – resistance.
I – reduced sensitivity.

S – wrażliwość.
R – oporność.
I – zmniejszona wrażliwość.

the strains and transconjugants were sensitive to AK, CI, and TS and revealed significant resistance to nalidixic acid (Table 3).

Moreover, the interpretation of the evaluated *Salmonella* sensitivity to fluorochinolones is controversial. Contrary to the advice of E-test manufacturers, it is currently assumed that an MIC for ciprofloxacin in the range of 0.125–0.5 mg/l proves a decreased sensitivity to this antibiotic [29]. Consistent with this suggestion, evaluation of *Salmonella* sensitivity to fluorochinolone antibiotics requires the determination of the MIC. The drug sensitivity findings obtained by Hanaken et al. [30] demonstrated that 94% of *Salmonella* strains resistant to nalidixic acid revealed a mutation in the gene *gyrA*, which resulted in decreased sensitivity to ciprofloxacin.

Considering the fact that more and more bacterial strains resistant to the majority of available drugs are observed, the possibility of inhibiting the process of gene transmission by conjugation seems very important. A number of reports describing such an activity of drugs or enzymes has been published recently. Leite et al. [31] demonstrated an inhibitory effect of enzymes obtained from macerated papaya seeds on conjugational transfer of R plasmid from an *S*. Typhimurium strain to *E. coli* cells. A 71% reduction in the number of transconjugants compared with a control (100%) was demonstrated in experiments using the enzymes *in vitro* and *in vivo* on mice [31]. Perhaps a wider use of such substances as supplements to antibiotic therapy could result in inhibiting the development of antibiotic resistance.

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