JOURNAL OF FOOD QUALITY & HAZARDS CONTROL
SHAHID SADOUGHI UNIVERSITY OF MEDICAL SCIENCES & HEALTH SERVICES

Journal of Food Quality and Hazards Control 2 (2015) 51-55

Antibiotic Resistance of Enterobacteriaceae Isolated from The Domestic Food Related Environments

I. Azevedo, H. Albano, J. Silva, P. Teixeira*

CBQF-Centro de Biotecnologia e Química Fina–Laboratório Associado, Escola Superior de Biotecnologia, Universidade Católica Portuguesa/Porto, Rua Arquiteto Lobão Vital, Apartado 2511, 4202-401 Porto, Portugal

Article type
Original article

Keywords
Anti-Bacterial Agents
Enterobacteriaceae
Environment
Food Safety

Received: 6 Nov 2014
Revised: 1 Dec 2014
Accepted: 28 Dec 2014

Abstract

Background: Multidrug resistant Enterobacteriaceae which was confined to the hospital environments is now emerging in the domestic food related environments as well. The main objective of the present study was to investigate the prevalence of antibiotic resistant Enterobacteriaceae in the domestic food related environments.
Methods: Resistance to ampicillin, chloramphenicol, ciprofloxacin, gentamicin, tetracycline, nalidixic acid, nitrofurantoin, and trimethoprim was evaluated in 125 isolates; collected in domestic food related environments using agar micro dilution method.
Results: Results indicated that 49.6% of the isolates were resistant to at least one antibiotic (32.8% to ampicillin, 6.4% to nitrofurantoin, 4% to tetracycline, 3.2% to nalidixic acid, 2.4% to chloramphenicol and 1.7% to trimethoprim). Resistance to multiple antibiotics was observed in 6.4% of the isolates.
Conclusion: This study implicates existence of antibiotic resistant Enterobactericeae in the domestic food related environments. This resistance phenomenon requires continual vigilance; and further studies are required to evaluate the role of domestic surfaces in the transmission of resistant pathogens and spread of infectious diseases.

Introduction

In the past decade, multidrug-resistant Enterobacteriaceae (MDE) has become an important challenge to disease control (Wellington et al., 2013). Gram-negative Enterobacteriaceae may cause severe infections and unfortunately several of the most important members of this family are becoming progressively more resistant to currently available antimicrobials (Denton, 2007; Fritsche et al., 2005; Paterson, 2006). Considering their wide spread and few options for treatment, MDE have public health importance (Khan et al., 2012). Resistance related to production of extended-spectrum β-lactamases (ESBLs) is a major concern about Enterobacteriaceae, but other ways of resistance are also existed, leading to multidrug resistance and creating pan-resistant bacteria strains (Paterson, 2006). ESBL-producing Enterobacteriaceae are usually found in the hospital setting, but it has been recently shown that they are prevalent in the community, too. The most significant reservoir of MDE is the intestine of human and domestic animals, especially in those who are frequently receiving antibiotics. The contamination of water, food, and the environment with MDE is an important route for its spread, whether from man or animals, and is therefore a crucial area for control (Pitout et al., 2005).

According to available data, numerous (infectious) diseases are obtained from the home environment (Beumer

*Corresponding author
E-mail: pcteixeira@porto.ucp.pt

[Downloaded from jfqhc.ssu.ac.ir on 2026-11-21]

and Kusumaningrum, 2003). In North America and Europe, more than 50% of the recorded food infections seem to be originated in the home (Azevedo et al., 2014; Beumer and Kusumaningrum, 2003).

Resistant microorganisms often emerge when the environment changes; thus, it is possible to emerge new resistant pathogens. The need for improved hygiene to reduce the spread of antibiotic resistance is a major recommendation of working parties across Europe (Bloomefield and Scott, 2013), not only in hospital but also in the community (Shannon and French, 2004). However, there is little information available until now about enteric microorganisms isolated from domestic food related settings.

Consequently, a main goal of the present study was to investigate the prevalence of antimicrobial susceptibility in Enterobacteriaceae isolates in 125 samples collected from the domestic food related environment. The potential repercussions of these results in terms of microbiological safety, especially concerning the development and spread of antimicrobial resistance in the food chain, will be discussed.

Materials and methods

Origin of Enterobacteriacea bacterial isolates

One hundred and twenty-five isolates of Enterobacteriaceae obtained from our previous study (Azevedo et al., 2014), stored at -20 ºC in Tryptone Soya Agar (TSA) with 30% (v/v) glycerol. They had been isolated from several points in 15 houses (from Oporto, Portugal), including knobs of doors, refrigerators and dishwashers, stove buttons, food preparation surfaces, taps and kitchen towels, as well as from domestic animals' feet that usually have access to the kitchen area, and WC knobs and taps.

Then, the isolates were cultured in Tryptone Soya Broth (TSB), for regeneration, and the purity of each culture was confirmed by streaking on plates of the respective medium.

Purified isolates were screened for Gram-negative, glucose positive, oxidase-negative and catalase-positive rea-ctions (Steel, 1961).

Antibiotic susceptibility testing

The minimum inhibitory concentrations (MIC; µg/ml) for each isolate were determined by the agar microdilution method, according to the Clinical and Laboratory Standards Institute (CLSI, 2007). Antibiotics were chosen on the basis of their diverse representation of different classes of antimicrobial agents. Each test was carried out on Muller-Hinton Agar (MHA) with cations adjusted for ampicillin (AMP) and on MHA for the seven other tested antibiotics including ciprofloxacin (CIP), chloramphenicol (CHL), gentamicin (GEN), nalidixic acid (NAL), nitrofurantoin (NIT), tetracycline (TET) and trimethoprim (TMP). With the exception of TMP ranging from 0.0156 to 128 µg/ml, all the other antibiotic concentrations ranged from 0.0156 to 512 µg/ml. After overnight cultures on TSA plates, the inocula were obtained by suspension in sterile Ringer's solution to achieve turbidity equivalent to 0.5 McFarland standards. Approximately 1 µl was positioned on each plate containing antibiotic with an automatic plating system (Mast Group, Ltd.).

All isolates were cultured on plates of MHA and MHA media with cations adjusted with no antibiotic as negative controls. The quality control strains *Enterococcus faecalis* ATCC 29212 and *Escherichia coli* ATCC 25922 were used to monitor the accuracy of MICs (CLSI, 2007).

Then, the plates were incubated overnight at 37 ºC. Susceptibility classification of isolates (as sensitive, intermediate or resistant) was carried out according to CLSI (2007).

To measure the susceptibility of each antibiotic, at least duplicate experiments were done. Bacterial isolates that revealed resistance to two or more antimicrobial agents of different classes were considered as multi-resistant isolates.

Results

As shown in Table 1, 49.6% of the isolates were resistant to at least one antibiotic, 32.8% to AMP. A low level of resistance to NIT (6.4%), TET (4.0%), NAL (3.2%), CHL (2.4%) and TMP (1.7%) was observed.

As it has been shown in Table 2, eight (6.4%) Enterobacteriaceae isolates were found to be resistant to more than one antibiotic, and four isolates were also resistant to more than three antibiotics. The Enterobacteriaceae isolates obtained from the kitchen cloth and dishwasher handle presented resistance to AMP, CHL, NAL and TET and the two cutting board isolates also showed resistance to three and four antibiotics, respectively (Table 2).

[Downloaded from jfqhc.ssu.ac.ir on 2026-11-21]

Table 1: In vitro susceptibility of Enterobacteriaceae isolates to several antibiotics and minimum inhibitory concentration (MIC) breakpoints

Class	Antibiotic	MIC (µg/ml) breakpoints			Enterobacteriaceae isolates		
		(S) Sensitive	(I) Intermediate	(R) Resistant	Number of sensitive isolates (%)	Number of intermediary isolates (%)	Number of resistant isolates (%)
Penicillins	AMP	≤8	16	≥32	68 (54.4)	16 (12.8)	41 (32.8)
Phenicols	CHL	≤8	16	≥32	117 (93.6)	5 (4.0)	3 (2.4)
Fluoroquinolones	CIP	≤1	2	≥4	125 (100)	*	*
Aminoglycosides	GEN	≤4	8	≥16	125 (100)	*	*
Tetracyclines	TET	≤4	8	≥16	114 (91.2)	6 (4.8)	5 (4.0)
Quinolones	NAL	≤16	---	≥32	121 (96.8)	*	4 (3.2)
Folate pathway inhibitor	TMP	≤8	---	≥16	124 (99.2)	*	1 (1.7)
Nitrofurantoins	NIT	≤32	64	≥128	86 (68.8)	31 (24.8)	8 (6.4)

*Blank spaces indicate that no MIC value was determined for that concentration

Table 2: Number of multi-resistant isolates and place of isolation

Antibiotics	Kitchen counter	Stove buttons	Dishwasher handle	Kitchen cloth	Cutting board
AMP, TET, NIT					1
AMP, CHL, TET, NIT					1
AMP, NIT	2			1	
AMP, CHL, NAL, TET			1	1	
AMP, TET		1			

Discussion

Enterobacteriaceae isolates were collected from different places of 10 houses and they were distributed all around the house with high isolation rates (Azevedo et al., 2014). As mentioned, 49.6% of the isolates were found to be resistant to at least one antibiotic. Little data has been reported about antibiotic resistance in Enterobacteriaceae isolates found in the domestic settings (Wellington et al., 2013). Enteric bacteria which originated from man and animal reservoirs can contaminate the domestic environments through cross-contamination. In animals as in humans, misuse of antibiotics may not only cause an increase of resistance in pathogenic bacteria, but also in the endogenous flora of these animals. Resistant bacteria from these animals may be transferred to the human population not only by direct contact but also through food products of animal origin. These resistant bacteria may then either colonize humans and/or transfer their resistance genes to other bacteria in the human intestinal flora (Van den Bogaard et al., 2000).

As observed, AMP was the antibiotic that showed more resistant Enterobacteriaceae isolates (32.8%), which is not surprising, since semi-synthetic penicillins (e.g., AMP and carbenicillin) were introduced in 1960s, generally now being ineffective against some bacteria, that were acquiring resistance, especially Enterobacteriaceae (Wellington et al., 2013).

A low level of resistance was found for NIT (6.4%), TET (4.0%), NAL (3.2%), CHL (2.4%) and TMP (1.7%). For more than 50 years NIT has been an option for the management of urinary tract infection, but its use declined with the introduction of alternative antimicrobials, like TMP (Hooton and Stamm, 1997). Isolates of the Enterobacteriaceae family from chicken, pork, fish and even water have shown worldwide TMP resistance (Schwaiger et al., 2012; Su et al., 2011; Tao et al., 2010). Some antibiotics such as TMP has been the core therapy for urinary tract infection for the past many years with a 90% success rate as the first-line agent indicated for the treatment of acute urinary tract infection and pyelonephritis (Hooton et al., 1995; Paterson, 2006). As already stated, there is few information about presence of MDE in the domestic settings, but we can compare with some other antibiotic resistance studies where Enterobacteriaceae were isolated from food products: isolates from milk products presented no resistance to NAL but some resistance was detected for TET (14.28%) and CHL (9.52%); among samples of cheese, 24% of Enterobacteriaceae isolates were resistant to TET but no resistance was found for CHL and NAL; and some dairy products showed high rates of resistance to TET (52.38%), but also no resistance was found to CHL and NAL (Hleba et al., 2011).

[Downloaded from jfqhc.ssu.ac.ir on 2026-11-21]

In our study, all the examined isolates were sensitive to GEN and CIP. This finding is similar to the some previous investigations in other regions carried out in non-domestic environment (Fluckey et al., 2007; Knezevic and Petrovic, 2008; Miranda et al., 2008). Even though resistance to fluoroquinolones (CIP) was not observed in this present study, over the past decade the emergence of high-level, fluoroquinolone resistance among *E. coli* and other species of Enterobacteriaceae has been recorded (Paterson, 2006).

Resistance to more than one antibiotic was verified for eight (6.4%) Enterobacteriaceae isolates, with four isolates resistant to more than three antibiotics. It should be noted that only those MDE isolated from the kitchen revealed resistance to more than one antibiotic. The isolates of kitchen cloth and dishwasher handle showed resistance to CHL, AMP, TET and NAL and the two isolates of cutting board also revealed resistance to three and four antibiotics, respectively (Table 2). This matter could be also justified by the contact with more raw foods, including raw meat, since as known, in animal food production, antibiotics used for disease treatment, disease prevention (prophylaxis) and growth promotion. Several investigators indicated that the occurrence of antibiotic-resistant bacteria in food of animal origin is high in developing countries, may be due to the inappropriate antibiotic therapy in animal farming (Van et al., 2012).

Conclusion

This study illustrates that Enterobacteriaceae showed low resistance profiles to frequently used antimicrobial agents (exception to AMP), but some of them, showed multi-resistance profiles. Therefore, antibiotic resistance including multi-resistance is not restricted to hospital environments, since such strains can also be found in the domestic food related environment. It is necessary to find procedures to control the spread of resistance by pathogens in the Enterobacteriacea in order to avoid this resistance phenomenon. The upsurge in multidrug-resistant strains of Enterobacteriaceae during the past decade is threatening the successful treatment of infections caused by these bacteria.

Conflicts of interest

None declared.

Acknowledgments

This work was supported by National Funds from FCT - Fundação para a Ciência e a Tecnologia through project PEst-OE/EQB/LA0016/2013. Financial support for authors Helena Albano and Joana Silva was provided by postdoctoral fellowships, SFRH/BPD/47826/2008 and SFRH/BPD/35392/2007, respectively (FCT–Fundação para a Ciência e a Tecnologia). Editing of this manuscript by Dr P.A. Gibbs is gratefully acknowledged.

References

Azevedo I., Albano H., Silva J., Teixeira P. (2014). Food safety in the domestic environment. *Food Control*. 37: 272-276.

Beumer R.R., Kusumaningrum H. (2003). Kitchen hygiene in daily life. *International Biodeterioration and Biodegradation*. 51: 299-302.

Bloomefield S.B., Scott E.A. (2013). A risk assessment approach to use of antimicrobials in the home to prevent spread of infection. *American Journal of Infection Control*. 41: S87-S93.

Clinical and Laboratory Standards Institute (CLSI). (2007). Performance standards for antimicrobial susceptibility testing; seventeenth informational supplement. pp: 98-141.

Denton M. (2007). Enterobacteriacae. *International Journal of Antimicrobial Agents*. 29: S9-S22.

Fluckey W.M., Loneragan W.G., Warner R., Brashears M.M. (2007). Antimicrobial drug resistance of Salmonella and *Escherichia coli* isolates from cattle feces, hides, and carcasses. *Journal of Food Protection*. 70: 551-556.

Fritsche T.R., Strabala P.A., Sader H.S., Dowzicky M.J., Jones R.N. (2005). Activity of tigecycline tested against a global collection of Enterobacteriaceae, including tetracycline-resistant isolates. *Diagnostic Microbiology and Infectious Disease*. 52: 209-213.

Hleba L., Kačániová M., Pochop J., Lejková J., Čuboň J., Kunová S. (2011). Antibiotic resistance of Enterobacteriaceae genera and *Salmonella* spp., *Salmonella enterica* ser. Typhimurium and Enteriditis isolated from milk, cheese and other dairy products from conventional farm in Slovakia. *Journal of Microbiology, Biotechnology and Food Sciences*. 1: 1-20.

Hooton T.M., Stamm W.E. (1997). Diagnosis and treatment of uncomplicated urinary tract infection. *Infectious Disease Clinics of North America*. 11: 551-581.

Hooton T.M., Winter C., Tiu F., Stamm W.E. (1995). Randomized comparative trial and cost analysis of 3-day antimicrobial regimens for treatment of acute cystitis in women. *Journal of The American Medical Association*. 273: 41-45.

Khan A.S., Dancer S.J., Humphreys H. (2012). Priorities in the prevention and control of multidrug-resistant Enterobacteriaceae in hospitals. *Journal of Hospital Infection*. 82: 143-151.

Knezevic P., Petrovic O. (2008). Antibiotic resistance of commensal *Escherichia coli* of food-producing animals from three Vojvodinian farms, Serbia. *International Journal of Antimicrobial Agents*. 31: 360-363.

Miranda J.M., Guarddon M., Va´zquez B.I., Fente C.A., Barros-Vela´zquez J., Cepeda A., Franco C.M. (2008). Antimicrobial resistance in Enterobacteriaceae strains isolated from organic chicken, conventional chicken and conventional turkey meat: a comparative survey. *Food Control*. 19: 412-416.

Paterson D.L. (2006). Resistance in Gram-negative bacteria: Enterobacteriaceae. *The American Journal of Medicine*. 119: S20–S28.

Pitout J.D., Nordmann P., Laupland K.B., Poirel L. (2005). Emergence of Enterobacteriaceae producing extended-spectrum β-lactamases (ESBLs) in the community. *Journal of Antimicrobial Chemotherapy*. 56: 52–59.

Schwaiger K., Huther S., Hölzel C., Kämpf P., Bauer J. (2012). Prevalence of antibiotic-resistant Enterobacteriaceae isolated from chicken and pork meat purchased at the slaughterhouse and at retail in Bavaria, Germany. *International Journal of Food Microbiology*. 154: 206-211.

Shannon K.P., French G.L. (2004). Increasing resistance to antimicrobial agents of Gram-negative organisms isolated at a London teaching hospital, 1995-2000. *Journal of Antimicrobial Chemotherapy*. 53: 818-825.

[Downloaded from jfqhc.ssu.ac.ir on 2026-11-21]

Steel K.J. (1961). The oxidase reaction as a taxonomic tool. *Journal of General Microbiology*. 25: 297-306.

Su H.C, Ying G.G., Tao R., Zhang R.Q., Fogarty L.R., Kolpin D.W. (2011). Occurrence of antibiotic resistance and characterization of resistance genes and integrons in Enterobacteriaceae isolated from integrated fish farms in South China. *Journal of Environmental Monitoring*. 13: 3229-3236.

Tao R., Ying G.G., Su H.C., Zhou H.W., Sidhu J.P. (2010). Detection of antibiotic resistance and tetracycline resistance genes in Enterobacteriaceae isolated from the Pearl rivers in South China. *Environmental Pollution*. 158: 2101-2109.

Van T.T.H., Nguyen H.N.K., Smooker P.M., Colo P.J. (2012). The antibiotic resistance characteristics of non-typhoidal *Salmonella enterica* isolated from food-producing animals, retail meat and humans in South East Asia. *International Journal of Food Microbiology*. 154: 98-106.

Van den Bogaard A.E., London N., Stobberingh E.E. (2000). Antimicrobial resistance in pig faecal samples from the Netherlands (five abattoirs) and Sweden. *Journal of Antimicrobial Chemotherapy*. 45: 663-671.

Wellington E.M.H., Boxall A.B.A., Cross P., Feil E.J., Gaze W.H., Hawkey P.M., Johnson-Rollings A.S., Jones D.L., Lee N.M., Otten W., Thomas C.M., Williams A.P. (2013). The role of the natural environment in the emergence of antibiotic resistance in Gram-negative bacteria. *The Lancet Infectious Diseases*. 13: 155-165.

[Downloaded from jfqhc.ssu.ac.ir on 2026-11-21]