Carbapenemase-producing bacteria in food-producing animals, wildlife and environment: A challenge for human health

Silvia Bonardi, Rosario Pitino
Department of Veterinary Science, University of Parma, Italy

Abstract

Antimicrobial resistance is an increasing global health problem and one of the major concerns for economic impacts worldwide. Recently, resistance against carbapenems (doripenem, ertapenem, imipenem, meropenem), which are critically important antimicrobials for human cares, poses a great risk all over the world. Carbapenemases are β-lactamases belonging to different Ambler classes (A, B, D) and encoded by both chromosomal and plasmidic genes. They hydrolyze a broad variety of β-lactams, including carbapenems, cephalosporins, penicillins and aztreonam. Despite several studies in human patients and hospital settings have been performed in European countries, the role of livestock animals, wild animals and the terrestrial and aquatic environment in the maintenance and transmission of carbapenemase-producing bacteria has been poorly investigated. The present review focuses on the carbapenemase-producing bacteria detected in pigs, cattle, poultry, fish, mollusks, wild birds and wild mammals in Europe as well as in non-European countries, investigating the genetic mechanisms for their transmission among food-producing animals and wildlife. To shed light on the important role of the environment in the maintenance and genetic exchange of resistance determinants between environmental and pathogenic bacteria, studies on aquatic sources (rivers, lakes, as well as wastewater treatment plants) are described.

Introduction

The resistance against carbapenems is of great public concern, because they are among the most critically important antimicrobials for human treatments (WHO, 2017). In fact, these antimicrobials have the broadest spectrum of activity in vitro against Gram-positive and Gram-negative bacteria, including anaerobes (Zhanel et al., 2007). Hence, they are often used as “last-line agents” or “antibiotics of last resort” to treat infections caused by multidrug-resistant Gram-negative bacteria (GNB) (Zhanel et al., 2007; Nordmann et al., 2011; Patel and Bonomo, 2013). In order to preserve their efficacy, the use in animal species should be banned (WHO, 2015). Carbapenems (biapenem, doripenem, ertapenem, faropenem, imipenem, meropenem, panipenem) are potent members of the β-lactam family and occupy a unique position among β-lactams because they are resistant to most β-lactamases from Gram-positive and Gram-negative bacteria. In fact, even if carbapenems share a penicillin-like ring with penicillins and cephalosporins, they possess a carbon instead of a sulfone in the fourth position of the thiazolidinonic moiety of the β-lactam ring (Kattan et al., 2008). Carbapenems are very efficient to treat a wide variety of infections such as complicated intra-abdominal infections, skin and skin structure infections, community-acquired and nosocomial pneumonia, complicated urinary tract infections, meningitis (meropenem only) and febrile pneumonia (Janssen et al., 2015). Furthermore, the use of carbapenems is rising in humans in response to the increase of Extended-Spectrum Beta-Lactamase (ESBL)-producers and other multiresistant bacteria (Ashiru-Oredope et al., 2012). All clinically available carbapenems have low oral availability because they do not cross the gastrointestinal barrier readily but must be administered intravenously; however, the combination imipenem-cilastatin and ertapenem can also be delivered intramuscularly (Papp-Walace et al., 2011). Since the use of carbapenems has never been licensed for food-producing animals in any country worldwide (WHO, 2015), their residues in foods of animal origin are not actually allowed. Among microorganisms involved in the antimicrobial resistance (AMR) phenomenon, GNB are becoming increasingly resistant to most antibiotics (Blair et al., 2014). Specifically, this trend is highlighted in Enterobacteriaceae, whose members are often involved into a wide variety of community and health-care infections (Grundmann et al., 2010; Nordmann et al., 2011; Albiger et al., 2015; Grundmann et al., 2017; Magiorakos et al., 2017). However, during the last years, several studies have shown the worldwide increase, in terms of prevalence, of carbapenem resistance in non-fermenting microorganisms, such as Pseudomonas spp. and Acinetobacter spp. (Miriaou et al., 2010; Pfeifer et al., 2010; Papp-Walace et al., 2011; EFSA, 2013).

The main mechanism of resistance to β-lactams among GNB is due to the presence of β-lactamases. Among over 1,000 of naturally occurring β-lactamases, carbapenemases with either serine-based or zinc-facilitated hydrolysis mechanisms are posing some of the most critical problems (Bush and Fisher, 2011). Among GNB, Carbapenem-Producing Enterobacteriaceae (CPE) are arising as an important challenge in health-care settings all over the world (Bush and Fisher, 2011; Nordmann et al., 2011) especially because they can acquire resistance by horizontal transfer of genes carried by mobile genetic elements (Woodford et al., 2014).

As well-known, the use of antimicrobials leads to a selective pressure for resistant strains both in humans and animals (Woodford et al., 2014). For instance, the detection of carbapenem-resistant bacteria in food-producing animals may be due to the use of extended-spectrum cephalosporins on farm. The exact relationship between extended-spectrum cephalosporin use and carbapenem-resistance has not fully been established (Mollenkopf et al., 2017), even if plasmids carrying genes conferring both resistance to carbapenems (bla_VIM) and third-generation cephalosporins (blaACC,1) have been recently found in bacteria isolated from livestock (Falgenhauer et al., 2017). This finding needs further studies, since administration of these drugs is commonly reported when carbapenem-resistance microorganisms are found in...
food-producing animals. This short review is focused on the occurrence of carbapenemase-producing (CP) bacteria in food-producing animals, natural environment and wildlife and its major goal is to trace their spreading and evolution out of the human compartment. Only studies which demonstrated the occurrence of CP bacteria out of the human compartment are described. Even if data from food-producing animals and environmental sources are fragmentary and often their epidemiological links seem to be not clear, our efforts aimed to select the studies which clearly described i) the analytical methods used; ii) the genetic determinants for carbapenem-resistance; iii) the possible links between use of antimicrobials in livestock and selection of CP bacteria. When available, data on co-resistance to other antimicrobials and their genetic mechanisms of transmission are reported.

Materials and Methods

A systematic review of 308 studies published in the PubMed database between 1980 and 2018 was performed, with special interest for those reporting the detection of CP bacteria in food-producing animals, wildlife and the environment. We included three types of articles: i) the articles describing carbapenem-resistant bacteria isolated from livestock (pigs, cattle, poultry) and products thereof, included fish and molluscs, the natural environment and wild animals; ii) the articles describing the different classes and variants of carbapenemases shared by bacteria of human and animal origin; iii) the articles describing the genomic traits of CP microorganisms isolated from livestock, wild animals and the environment. Studies primarily focused on laboratory methods for detection of CP bacteria were excluded, as well as those regarding carbapenem-resistant bacteria from companion animals (dogs and horses) or insects (flies).

Among the articles found by the entry criteria, only those showing data on CP bacteria and bacterial carriage of carbapenemase genes were included in the review, thus excluding all studies focused on bacterial resistance to carbapenems not based on carbapenemases production.

Development of carbapenem-resistance

Bacteria show resistance to carbapenems through several mechanisms: production of β-lactamases (carbapenemases), efflux pumps and mutations that alter the expression and/or function of porins and penicillin-binding proteins (PBPs). CP microorganisms are usually only susceptible to polymyxins (e.g. colistin), fosfomycin and variably susceptible to tigecycline, although colistin resistance in CP Klebsiella pneumoniae isolates has been recently reported (Nordmann et al., 2009; Pena et al., 2014; Otter et al., 2017).

Still other mechanisms can be active, the production of specific β-lactamases, called carbapenemases, is the most important to confer resistance against carbapenems. In fact, carbapenemases are able to hydrolyze carbapenems efficiently, while most other β-lactamases hydrolyze them very slowly (Temkin et al., 2014). Furthermore, carbapenemases hydrolyze a broad variety of β-lactams, including cephalosporins, penicillins and aztreonam and are inhibited by the β-lactamase inhibitors, such as clavulanic acid, tazobactam and sulbactam (Drazw and Bonomo, 2010).

Carbapenemases have been classified in three classes: the Ambler class A, B and D β-lactamases (Ambler, 1980). Ambler class C β-lactamases, on the contrary, possess a slightly extended activity towards carbapenems, but primarily hydrolyze cephalosporines (AmpC) (Jaurin and Grundstrom, 1981). Based on their hydrolytic activity, carbapenemases are divided into two groups: i) serine carbapenemases, which utilize the amino acid serine for β-lactam hydrolysis by forming an acyl enzyme (Class A and D); ii) metallo carbapenemases, which require at least one active-site zinc ions to facilitate β-lactam hydrolysis (Class B) (Hall and Barlow, 2005).

Class A carbapenemases can be chromosomally encoded, such as NmcA (Nonmetallo carbapenemase A), SME (Serratia marcescens enzyme), IMI-1 (Imipenem-hydrolyzing β-lactamase), SFC-1 (Serratia fonticola carbapenemase), BIC-1 (Bicêtre carbapenemase), PenA (penicillinase from P. cepacia), FPH-1 (from Francisella philomiragia) and several members of SHV family, or plasmid encoded, such as KPCs (Klebsiella pneumoniae carbapenemases), GES (Guiana extended spectrum β-lactamase), IMI-2, and FRI-1 (Aubron et al., 2005; Naas et al., 2016). Chromosomally mediated AMR is vertically transmitted to daughter cells, and these microorganisms can be clinically relevant if they produce severe infections requiring antimicrobial therapy. However, bacterial β-lactamase genes located on mobile plasmids pose a far greater health threat because they may be transmitted horizontally among commensal bacterial and pathogens, thus potentially disseminating β-lactam resistance to a great variety of bacterial species. For example, in 2001, evidence of horizontal gene transfer (CMY-2 AmpC β-lactamase) was demonstrated in food-producing animal and human isolates of E. coli and Salmonella resistant to cefamycins and third-genera cephalosporins (Winokur et al., 2001). Since then, other studies have observed the transfer of plasmid-mediated carbapenem resistance between different bacterial species (Goren et al., 2010; Rumbo et al., 2011; Hardiman et al., 2016). In addition, evidence for the transfer of CP gene-containing transposons between plasmids has been demonstrated, as the bla_KPC-2 gene in K. pneumoniae. The bla_KPC-2 gene is located on the Tn-3 related transposon Tn4401 capable of high frequency of transposition (Nordmann et al., 2009; Cuzon et al., 2011). The transposon Tn4401 has been also described in the plasmid pCOL-1 of P. aeruginosa, thus suggesting that it could be transferred among different microorganisms (Diene and Rolain, 2014).

KPCs are class A serine β-lactamases that spread primarily via the clonal dissemination of K. pneumoniae and some KPC-producing clones are dominant, such as the sequence type (ST) 258 (Cuzon et al., 2010), ST512 (Warburg et al., 2012) and ST11 (Cuzon et al., 2010; Baraniak et al., 2011). Beside the gene bla_KPC-2 (Nordmann et al., 2009; Cuzon et al., 2011), other bla_KPC genes are present on a wide variety of plasmids, different for size, structure and nature (Gootz et al., 2009; Cuzon et al., 2010; Leavitt et al., 2010). KPCs comprise 22 variants that differ by one to five amino acid substitutions (Woodford et al., 2014). As KPC-1 sequence was found to be identical to KPC-2, KPC-1 is no longer a valid designation (Vigil et al., 2008).

Among class B metallo β-lactamases (MBLs), the New Delhi metallo-β-lactamases (NDMs) and the Verona integron-encoded metallo-β-lactamases (VIMs) are the most common. NDM-producing species comprise E. coli (Mushfaq et al., 2011; Cuzon et al., 2013) and K. pneumoniae (Pitout et al., 2015) but also Acinetobacter spp. (Zhang et al., 2014) and Pseudomonas spp. (Walsh et al., 2011). Dissemination of blaNDM-1 occurs both by horizontal and vertical transfer, since the coding sequence may be found on different plasmids or located on the chromosomes (Poirel et al., 2011). To date, different variants of the NDM β-lactamases (NDM-1 to 7) have been identified in the Indian subcontinent (Rahman et al., 2014). VIM is an important MBL spread both in Enterobacteriaceae and non-fermenting bacteria. After the initial discovery of blaVIM-1 in a clinical isolate of P. aerugi-
Epidemiology of carbapenem-resistance among bacteria

Across the globe, first reports of carbapenemases occurred in the 1980s. For over 20 years there were no significant reports with an exception represented by a limited spread of IMP metallo-β-lactamase in *Aeromonas hydrophila* in Japan (Senda et al., 1996; Temkin et al., 2014). Afterwards, other cases were reported in London (1982) by Seoul imipenemase (SME-1) from *Serratia marcescens* (Yang et al., 1990), in California (1984) by imipenemase (IMI-1) from *Enterobacter cloacae* (Rasmussen et al., 1996) and in France (1990) by NMC-A from *Enterobacter cloacae* (Nordmann et al., 1993).

The most frequently carbapenemases associated with Enterobacteriaceae worldwide are those belonging to the KPC family (Woodford et al., 2011; Rimoldi et al., 2017; van Duin and Doi, 2017; Kim et al., 2018; Muggeo et al., 2018). In 2017, among the European Union (EU) and European Economic Area (EEA) countries, the highest detection rates for KPC-producing *Klebsiella pneumoniae* human invasive isolates were reported from Greece (64.7%), Italy (29.7%) and Romania (22.5%), where the resistance situation for carbapenemases remains problematic. On the contrary, very low to low prevalence (0.1-1.7%) were generally notified from northern and western European countries. Overall, the EU/EEA population weighted mean percentage for *K. pneumoniae* was 7.2% in 2017 (ECDC, 2018). Since resistance to carbapenemases in *K. pneumoniae* is mediated by a wide range of carbapenemases, such as KPC, NDM, OXA-48-like and VIM, although with wide variation in prevalence (Grundmann et al., 2017), CP *K. pneumoniae* strains may show resistance to all available β-lactams. Additionally, high percentage of isolates have co-resistance to fluoroquinolones, third-generation cephalosporsins and aminoglycosides (ECDC, 2018).

Carbenapenem-resistance among human invasive *Escherichia coli* strains was still reported to be rare in all EU/EEA countries, with a population weighted mean percentage of 0.1% in 2017 (ECDC, 2018). Carbenapenem-resistance combined with resistance to other important antimicrobial groups was commonly reported in *P. aeruginosa* in many EU/EEA countries in 2017, with a weighted mean of 17.4% (ECDC, 2018). Unlike other bacteria, carbapenem-resistance in *P. aeruginosa* is multifactorial, due to plasmid or integron-coded carbapenemases, increased expression of efflux systems, reduced porin expression and increased chromosomal cephalosporinase activity (Meletis et al., 2012). Regarding *Acinetobacter baumannii*, the epidemiological situation for carbapenem-resistance strains has been worsening in several European countries. Like *K. pneumoniae* and *E. coli*, carbapenem-resistance in *A. baumannii* is often mediated by carbapenemases production. In 2017, several European countries reported high percentages of resistance against carbapenemases in *Acinetobacter spp.*, which ranged from 0.0% in some northern countries (Denmark, Norway and Sweden) to 96.2% (Croatia), southern and eastern countries, like Italy, Latvia, Bulgaria, Romania, Lithuania and Greece reported prevalence values of 78.7%, 79.4%, 80.4%, 87.4%, 88.5% and 94.8%, respectively (ECDC, 2018).

The so called ‘big five’ carbapenemase families collectively cause the greatest clinical concern. They include the KPC and OXA-48-like non-metallo-enzymes and the IMP, NDM and VIM metallo-enzymes (Nordmann et al., 2011; Patel and Bonomo 2013). There are significant country and regional differences in the importance of these families. In Europe, the KPC, OXA-48-like, NDM and VIM enzymes commonly dominate (Cantón et al., 2012), whereas IMP enzymes are more prevalent in the Far East and Australia (Chen et al., 2014). Furthermore, the genes responsible for resistance to carbapenemases are frequently associated with genes coding for resistance to non-β-lactam antimicrobials, giving rise to the so called multidrug resistant (MDR) microorganisms (Nordmann et al., 2011).

Food-producing animals and carbapenemase-producing bacteria

The occurrence of CP bacteria in food-producing animals has been reported from several countries. Pigs and chickens represent the most investigated species, in which carbapenem-resistance in Enterobacteriaceae and non-fermenting microorganisms (*Acinetobacter spp.* and *Pseudomonas spp.* has been most frequently observed. Different classes of carbapenemases have been found both in Enterobacteriaceae and non-fermenting bacteria carried by livestock animals (Patel and Bonomo 2013).

Pigs

The most important studies on the detection of CP bacteria in pigs are shown in Tables 1 and 2.

The first detection of CP microorganisms in this animal species dates back to
2011, when a VIM-1- producing E. coli strain was isolated from a pig farm holding 4,100 pigs in Germany (Fischer et al., 2012). It was resistant to penicillin, cephalosporins, cephaparin, and amoxicillin/clavulanic acid, but was susceptible to aztreonam and intermediate/susceptible to carbapenems (imipenem, ertapenem and meropenem). By PCR, the isolate was found to harbor both the Amp-encoding gene bla<sub>ACC-1</sub> and the carbapenemase-encoding gene bla<sub>TEM-1</sub>, located in a class 1 integron labelled In110. This integron had been previously identified in other enterobacteria, such as Klebsiella oxytoca and Enterobacter cloacae, as well as in Pseudomonas aeruginosa and Pseudomonas putida in Italy and Spain (Riccio et al., 2005; Tato et al., 2010). The German VIM-1-producing E. coli strain represented the first CP microorganism isolated from pigs in Europe, but its sequence type (ST88) had been previously identified also among chickens, cattle and humans in Germany (Fischer et al., 2012). Retrospectively, it was observed that the VIM-1-positive E. coli ST88 strain was

### Table 1. Carbapenemase-producing Enterobacteriaceae from livestock.

<table>
<thead>
<tr>
<th>Bacterial species</th>
<th>Carbapenemase genes</th>
<th>Additional resistant genes *</th>
<th>Animal species</th>
<th>Source</th>
<th>Year</th>
<th>Country</th>
<th>Reference</th>
</tr>
</thead>
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<tr>
<td>E. coli</td>
<td>bla&lt;sub&gt;TEM-1&lt;/sub&gt;</td>
<td>bla&lt;sub&gt;TEM-1&lt;/sub&gt;, aph(3')-Ib-cr, aadA2, aadA5, aac(3)-Ia, armA, qnrS1, floR, cmrA1, sul1, sul2, tet(A), tet(M), dfrA12</td>
<td>Faeces</td>
<td>2011</td>
<td>Germany</td>
<td>Fischer et al., 2012</td>
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<tr>
<td>K. pneumoniae</td>
<td>bla&lt;sub&gt;TEM-1&lt;/sub&gt;</td>
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<td>Faeces</td>
<td>2011</td>
<td>Germany</td>
<td>EFSA and ECDC, 2015</td>
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<td>bla&lt;sub&gt;TEM-1&lt;/sub&gt;</td>
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<td>Faeces</td>
<td>2016</td>
<td>Italy</td>
<td>Palis et al., 2017</td>
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<td>K. pneumoniae</td>
<td>bla&lt;sub&gt;TEM-1&lt;/sub&gt;</td>
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<td>Faeces</td>
<td>2015</td>
<td>China</td>
<td>He et al., 2017</td>
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<td>E. coli</td>
<td>bla&lt;sub&gt;TEM-1&lt;/sub&gt;</td>
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*When reported by the authors.
widely distributed in the fattening-pig farm tested in 2011, demonstrating that persistence and dissemination of CP microorganisms on farm was possible (Fischer et al., 2017). In 2011-2012, another German study revealed the occurrence of VIM-1-producing Salmonella Infantis from two pig farms and one broiler farm (Fischer et al., 2013a). The isolates carried both the gene bla_{ACC-1} and the gene bla_{VIM-1}; the bla_{VIM-1} gene was located on a class 1 integron harbored by a ~300kb IncH12 plasmid, which also carried the bla_{ACC-1} gene. The sequence type (ST32) and PFGE pattern of the isolates were shared with other S. Infantis strains of human, poultry and pig origin previously detected in Germany (Fischer et al., 2017). Noteworthy, S. Infantis is among the top five Salmonella serovars responsible for human salmonellosis in Europe, ranking in fourth place in 2017, after Enteritidis, Typhimurium and monophasic Typhimurium (EFSA and ECDC, 2018a) and responsible for severe cases of human salmonellosis, characterized by septicaemia and even death (Naas et al., 2011). As a consequence, the detection of carbapenemase-producing S. Infantis in pigs and poultry, which are considered its main reservoir, is of great concern (Hauser et al., 2012; EFSA, 2013; Hindermann et al., 2017).

Further studies revealed that in VIM-1-producing S. Infantis and VIM-1-producing E. coli, isolated on the same farms, the bla_{VIM-1} gene was located on the IncH12 plasmid, also found in human strains. The IncH12 plasmid in S. Infantis (pRH-R27) was a mosaic plasmid with high homology to the plasmids isolated from the human strains, while the IncH12 plasmid in E. coli (pRH-R178) was a deletion derivative of the pRH-R27 found in S. Infantis, suggesting that selective evolution of plasmids in the livestock environment is possible (Falgenhauer et al., 2017). Since carbapenemases are not used in animal husbandry in Germany, the source of the bla_{VIM-1} gene could be identified in the selective pressure caused by third-generation cephalosporins. In fact, pRH-R27 and pRH-R178 plasmids harbored a plasmid-encoded AmpC β-lactamase (bla_{ACC-1}) which confers resistance to third-generation cephalosporins (Falgenhauer et al., 2017).

As recently observed, the presence of genes encoding resistance to antimicrobials frequently used in swine production (e.g. sulfonamides, cephalosporins) might be favored by minimal selective concentrations of the agents (below almost up to 140-fold the MIC value). Therefore, even a very low drug concentration found in livestock environments and in treated humans and animals might be sufficiently high to select and maintain plasmids carrying resistance in the bacterial populations (Gullberg et al., 2014).

In 2015, mandatory monitoring for ESBL-/AmpC-/carbapenemase-producing E. coli was performed on caecal contents from 6,167 fattening pigs, 2,347 calves under one year of age and 10,679 meat samples thereof in 23 member states and two non-member states in Europe. As recommended by EUCAST (2015) a screening breakpoint for cefotaxime and/or ceftazidime (> 1 mg/L) was applied to screen for ESBL and AmpC-producers. Lower ECOFF breakpoints were applied for meropenem (> 0.125mg/L), imipenem (> 0.5 mg/L) and ertapenem (> 0.06 mg/L) to screen for CP E. coli. Regarding ESBL and AmpC producers, prevalence was high for the former (31.9% and 36.8% in pigs and calves, respectively) and low for the latter (9.75 and 4.8% in pigs and calves, respectively) (EFSA and ECDC, 2017b). One VIM-1 E. coli strain was reported from pigs by Germany, thus confirming persistence of VIM-1-producing E. coli in the German pig population for at least four years (Irrgang et al., 2016). In addition, a CP E. coli strain was isolated from pig meat in Belgium (EFSA and ECDC, 2017b).

Recently, the occurrence of bla\textsubscript{OXA-181} E. coli from two pigs reared in an Italian farm was reported. The two strains were not genetically related, belonging to sequence types ST359 and ST641. One of the isolates carried also the colistin-resistance gene mcr-1 and the aminoglycoside-resistance gene armA. The gene bla\textsubscript{OXA-181} was located on a 51.5-kb non-conjugative IncX3 plasmid and the mcr-1 gene on a 33.3-kb transferable IncX4 plasmid; both plasmids showed high similarity to human and animal ones, demonstrating that antimicrobial resistance plasmids are largely distributed in E. coli strains (Pulss et al., 2017).

The occurrence of plasmid-borne carbapenemase genes among Enterobacteriaceae in pigs was also reported in the USA. From environmental and faecal samples collected from swine farrowing and nursery barns, 18 (5.6%) bla\textsubscript{IMP-27}-harboring isolates belonging to the Enterobacteriaceae family (E. coli, Proteus spp., Morganella spp., Providencia spp., Citrobacter spp., Klebsiella spp.) were identified (Mollenkopf et al., 2017). The bla\textsubscript{IMP-27} is considered rare among the β-lactamase genes in the United States (Widmann et al., 2012). Positive selection pressure due to cephalosporins use at farm was related to the higher prevalence of bla\textsubscript{IMP-27} carrying isolates in the farrowing barn, compared to the nursery and finishing barns. In fact, cefotiofur was given to all piglets after birth (days 0 to 1) and to males at castration (day 5 to 7). In all the isolates, the bla\textsubscript{IMP-27} gene was located on IncQ1 plasmids of ~10 kb, whose presence in multiple bacterial species strongly suggested that they were mobilizable (Mollenkopf et al., 2017). IncQ are small (5.1 to 14.0 kb) plasmids able to replicate independently of their host, allowing to reach high copy numbers (Meyer, 2009). Even if IncQ plasmids are not self-transmissible, they can be mobilized by a type IV transporters provided by larger, self-transmissible, co-resident helper plasmids from incompatibility groups, including IncP, IncF, IncL, IncM, IncX, IncN, and IncW (Loftie-Eaton and

Table 2. Carbapenemase-producing non-Enterobacteriaceae from livestock.

<table>
<thead>
<tr>
<th>Bacterial species</th>
<th>Carbapenemase genes</th>
<th>Additional resistant genes *</th>
<th>Animal species</th>
<th>Source</th>
<th>Year</th>
<th>Country</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. baumannii</td>
<td>bla\textsubscript{VIM-1}</td>
<td>aph\textsubscript{Ab}, ble, msr(E)-mph(E)</td>
<td>Pigs</td>
<td>Lungs of a diseased pig (pneumonia)</td>
<td>2011-2012</td>
<td>China</td>
<td>Zhang et al., 2013</td>
</tr>
<tr>
<td>Acinetobacter (related to A. lucii)</td>
<td>bla\textsubscript{OXA-23}</td>
<td></td>
<td>Dairy cattle</td>
<td>Faeces</td>
<td>2010</td>
<td>France</td>
<td>Poirel et al., 2012</td>
</tr>
<tr>
<td>A. baumannii</td>
<td>bla\textsubscript{OXA-47}</td>
<td></td>
<td>Dairy cattle</td>
<td>Faeces</td>
<td>2014</td>
<td>New Mexico and Texas, USA</td>
<td>Webb et al., 2016</td>
</tr>
<tr>
<td>A. lucii</td>
<td>bla\textsubscript{VIM-1}</td>
<td>aph\textsubscript{Ab}</td>
<td>Chickens</td>
<td>Faeces</td>
<td>2010</td>
<td>China</td>
<td>Wang et al., 2012</td>
</tr>
</tbody>
</table>

*When reported by the authors.
Rawlings, 2012). An experimental study evaluated the use of amoxicillin and ertapenem in porcine models; each antimicrobial resulted in changes in the porcine gut microbiome causing elimination of key commensal bacteria and propagation of AMR genes, including β-lactamases. Specifically, amoxicillin promoted the selection of many AMR genes, efflux pumps and β-lactamases, while ertapenem triggered the emergence of genes encoding for β-lactamases and the \( \text{bla}_{\text{OXA-23}} \) carbapenemase (Connelly et al., 2018). Besides this, other agents used in swine production, as heavy metals and disinfectants, could co-select for plasmids carrying resistance genes when there is no direct selective pressure (Johnson, 2017).

In South China a large survey on lungs, liver and lymph nodes of pigs, chickens and ducks was performed in 2011-2012. One \( \text{bla}_{\text{NDM}} \), \( \text{A. baumannii} \) isolate was detected in the lungs of a swine with pneumonia and sepsis, reared on a farm where different β-lactams, third- and fourth-generation cephalosporins (ceftaxime and cefotiuor), aminoglycosides and quinolones were commonly administered to the pigs. This evidence suggests for selective pressure favoring the emergence of carbapenem-resistance among microorganisms. The \( \text{bla}_{\text{OXA-1}} \) gene in \( \text{A. baumannii} \) was identified on a ~47 kb transferable plasmid (Zhang et al., 2013). At last, a worrisome finding comes from India and concerns the detection of Shiga-toxin producing \( \text{E. coli} \) (STEC) carrying \( \text{bla}_{\text{NDM-5}} \). Intriguingly, the genetic context of the \( \text{bla}_{\text{NDM-5}} \)-harboring \( \text{K. pneumoniae} \) isolates from cattle, but \( \text{bla}_{\text{OXA-23}} \)-carrying \( \text{K. pneumoniae} \) from cattle, \( \text{bla}_{\text{NDM-5}} \)-harboring \( \text{E. coli} \) and \( \text{bla}_{\text{NDM-5}} \)-carrying \( \text{K. pneumoniae} \) had been previously isolated from human patients in several countries, including the UK (Hornsey et al., 2011), Singapore (Balm et al., 2013), Algeria (Sassi et al., 2014), Japan (Nakano et al., 2014), Denmark (Hammerum et al., 2015), The Netherlands (Bathoorn et al., 2015), Spain (Pittard et al., 2015), the USA (de Man et al., 2015), Australia (Wailan et al., 2015), South Korea (Cho et al., 2015), India (Krishnaraju et al., 2015) and China (Liu et al., 2016; Zhang et al., 2016). In the Chinese dairy farms, selective pressure due to β-lactams (amoxicillin, cefotiuor) used to treat cattle with mastitis could have favored the emergence of \( \text{bla}_{\text{NDM-5}} \)-harboring \( \text{K. pneumoniae} \) isolates. In all the isolates, the \( \text{bla}_{\text{NDM-5}} \) gene was found on a ~46 kb self-transferable plasmid, named IncX3 pNDM-MGR194-like plasmid. Furthermore, since the strains belonged to five different sequence types, clonal dissemination within the farms could be only partly responsible of their widespread. Interestingly, the genetic context of the IncX3 plasmid was nearly the same of a human \( \text{K. pneumoniae} \) plasmid designated pNDM-MGR194, previously reported in India, and also closed to \( \text{bla}_{\text{NDM-5}} \)-carrying plasmids harbored by human \( \text{E. coli} \) strains in China (He et al., 2017). Most of these isolates were recovered from human clinical specimens and one isolate of \( \text{bla}_{\text{NDM-5}} \)-\( \text{E. coli} \) from a dog in Algeria (Yousfi et al., 2015). These evidences seem to support for transmission of IncX3 plasmids from human sources to animal ones. Recently, the \( \text{bla}_{\text{NDM}} \) genes (\( \text{bla}_{\text{NDM-1}} \) and \( \text{bla}_{\text{NDM-4}} \)) have frequently been reported to be located on IncX3 plasmids among several species of Enterobacteriaceae (\( \text{E. coli} \), \( \text{K. pneumoniae} \), \( \text{Citrobacter freundii} \) and \( \text{E. cloacae} \)) in China (Ho et al., 2012; Chen et al., 2016) implying that IncX3 plasmids may provide an efficient vehicle for dissemination of human \( \text{bla}_{\text{NDM}} \) genes within bacterial strains of animal origin. Therefore, the role of the IncX3 plasmid in the spread of the \( \text{bla}_{\text{NDM-5}} \) gene (maybe of human origin) in the Chinese dairy farms was suggested and opened options to re-transmission of \( \text{bla}_{\text{NDM-5}} \)-carrying plasmid/bacteria from animals to humans through the dairy food chain (He et al., 2017). This hypothesis seems to be confirmed by the occurrence of the IncX3 plasmid carrying the gene \( \text{bla}_{\text{NDM-5}} \) in \( \text{E. coli} \) strains isolated from teats and milk of dairy cows in Algeria (Yaici et al., 2016).

Poultry

The most interesting studies on detection of CP bacteria in poultry are shown in Tables 1 and 2.

Regarding EU countries, presumptive CP commensal \( \text{E. coli} \) isolates from broilers and boiler meat were reported by 2 member states in 2016 (11 isolates from Cyprus and 3 isolates from Romania). In Romania, the \( \text{E. coli} \) isolates from broilers were identified to carry the \( \text{bla}_{\text{OXA-46}} \) gene (EFSA and ECDC, 2018b).

Most studies on poultry have been performed in China and African countries. In China, one isolate of NDM-1-producing \( \text{A. baumannii} \) resistant to eight of nine β-lactams, including imipenem, meropenem and ertapenem, was detected from 396 anal swab samples collected from chickens. The gene \( \text{bla}_{\text{NDM-1}} \) was located on a ~270-kb self-transferable plasmid, designated pAL-01. Although the use of carbenapens in food-producing animals is banned in China, other β-lactams such as penicillin and cephalosporins (cefradine, cefotiuor and cefotaxime) are commonly used, as observed in the above-mentioned chicken farm (Wang et al., 2012).

Also, from China, a \( \text{bla}_{\text{OXA-23}} \) -harboring Salmonella isolate from a chicken carcass at slaughter was reported. The strain carried other β-lactamases, amynoglycosides, phenicol and trimethoprim/sulphamethoxazole resistance genes for a total of more than 20 antimicrobial resistances (Wang et al., 2017). The occurrence of such extensively-drug resistance (XDR) phenotype in the genus Salmonella is of the greatest concern for human health, due to its zoonotic attitude and widespread in the food chain.

High prevalence of \( \text{E. coli} \) carrying both resistance against colistin (gene \( \text{mcr}-1 \)) and carbenapens (genes \( \text{bla}_{\text{NDM-1}}, \text{bla}_{\text{NDM-4}}, \text{bla}_{\text{NDM-5}} \) and \( \text{bla}_{\text{NDM-8}} \)) was reported in broil-
ers by another Chinese study. In the isolates, dissemination of mcr-1 and blaNDM genes was not clonal, but due to different plasmids (Liu et al., 2017).

In Egypt, a study on carbapenem-resistant K. pneumoniae in broiler chickens reared in different farms, drinking water on farms and humans working in contact with chickens was performed. CP K. pneumoniae was isolated from 15% of broilers and 6% of water samples. Among the poultry isolates (n=15), all of them were blaNDM-positive, including 11 isolates harboring blaKPC, blaOXA-48 and blaNDM and four harboring either blaKPC and blaNDM or blaOXA-48 and blaNDM. The isolates from drinking water (n=3) were positive for blaKPC and blaNDM (n = 1) or for all three genes (n = 2). In Egypt, a high proportion (56%) of K. pneumoniae isolates from humans were positive for the three carbapenemase genes. This finding suggests that a high incidence of CP K. pneumoniae in humans may contribute to its dissemination among food-producing animals and the livestock environment, thus increasing the risk of foodborne transmission to the consumers (Hamza et al., 2016).

### Fish and molluscs

To prevent bacterial infections in farmed fish, antimicrobials are often used in intensive aquaculture practices (Rogers and Basurco, 2009). This situation supports the occurrence of AMR bacteria in seafood products (Roschanski et al., 2017). Furthermore, fish and molluscs may acquire AMR bacteria from water sea polluted by sewage and agriculture drains. Although investigations on CP bacteria in fishery products are very rare, some important data can be achieved from literature. The studies focused on the detection of CP bacteria in seafood are shown in Table 3.

In 2013, A. baumannii isolates harboring the chromosomally encoded blaOXA-51 gene and the acquired blaOXA-23 gene were detected from two Pagellus acarne fished in the Mediterranean Sea. The microorganisms were resistant to aminoglycosides, third-generation cephalosporins and carbapenems (imipenem, meropenem, ertapenem). They belonged to the widespread clone A. baumannii ST2, which had reached fish living in the Mediterranean Sea (Brahmi et al., 2016). Another study focused on CP bacteria contamination of frozen seafood (included octopus, squid, clams, mussels and shrimps) imported from China and Korea identified blaOXA-48-harboring Stenotrophomonas maltophilia, Pseudomonas putida and Myroides odoratimimus isolates (Morrison and Rubin, 2015).

Recently, a strain of VIM-1 producing E. coli ST10 has been isolated from Venus clams (Ruditapes philippinarum) harvested in the Italian Mediterranean Sea, suggesting that CP E. coli have reached the seafood chain of this country. Moreover, since Venus clams are often eaten raw, ideal conditions for transmission and spread to the consumers of CP bacteria and/or transfer of the respective plasmids could occur (Roschanski et al., 2017).

### Environment and carbapenemase-producing bacteria

The studies focused on the detection of CP bacteria from different environmental sources are shown in Table 4. Environmental contamination with antimicrobials, AMR bacteria and their transfer-

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### Table 3. Carbapenemase-producing Enterobacteriaceae and non-Enterobacteriaceae from fishery and wild animals.

<table>
<thead>
<tr>
<th>Bacterial species</th>
<th>Carbapenemase genes</th>
<th>Additional resistant genes *</th>
<th>Animal species</th>
<th>Source</th>
<th>Year</th>
<th>Country</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>E. coli</td>
<td>blaNDM</td>
<td>blaOXA-48, blaOXA-51, aacA4-like, aadA1, aph(3’)-I, aac(6’)-Ib, aac(3’)-I</td>
<td>Venus clams (Ruditapes philippinarum)</td>
<td>N.S.</td>
<td>2016</td>
<td>Mediterranean Sea (Italy)</td>
<td>Roschanski et al., 2017</td>
</tr>
<tr>
<td>A. baumannii</td>
<td>blaOXA-23, blaOXA-48</td>
<td>aac(3’)-Ib, aac(3’)-I</td>
<td>Fish (Pagellus acarne)</td>
<td>Gill's Gut</td>
<td>2013</td>
<td>Mediterranean Sea (Algeria)</td>
<td>Brahmi et al., 2016</td>
</tr>
<tr>
<td>S. maltophilia</td>
<td>blaOXA-48</td>
<td></td>
<td>Seafood medley **</td>
<td>Edible product</td>
<td>N.S.</td>
<td>China</td>
<td>Morrison and Rubin, 2015</td>
</tr>
<tr>
<td>M. odoratimimus</td>
<td>blaOXA-48</td>
<td></td>
<td>Clams</td>
<td></td>
<td></td>
<td>Korea</td>
<td></td>
</tr>
<tr>
<td>P. putida</td>
<td>blaOXA-48</td>
<td></td>
<td>Squid</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stenotrophomonas spp.</td>
<td>blaOXA-48</td>
<td></td>
<td>Sea squirt</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>S. Corvallis</td>
<td>blaOXA-48</td>
<td></td>
<td>Black kites (Mimus migrans)</td>
<td>N.S.</td>
<td>N.S.</td>
<td>Germany</td>
<td>Fischer et al., 2013</td>
</tr>
<tr>
<td>E. coli</td>
<td>blaNDM</td>
<td></td>
<td>Yellow-legged gulls (Larus michaelsii)</td>
<td>Faeces</td>
<td>2012</td>
<td>France</td>
<td>Vittecoq et al., 2017</td>
</tr>
<tr>
<td>E. coli</td>
<td>blaOXA-48</td>
<td>qacA, aacA4, catB3</td>
<td>Silver gulls (Chromis chromis)</td>
<td>Faeces</td>
<td>2012</td>
<td>Australia</td>
<td>Doleska et al., 2016</td>
</tr>
<tr>
<td>E. coli</td>
<td>blaOXA-48</td>
<td>qacA, aacA4, catB3</td>
<td>Wild boars (Sus scrofa)</td>
<td>Faeces</td>
<td>2016</td>
<td>Algeria</td>
<td>Bachiri et al., 2017</td>
</tr>
</tbody>
</table>

N.S., not specified; *when reported by the authors. **Seafood medley contains squid, octopus, mussels and shrimp.
able resistance genes can pose a serious threat not only to human health, but to the natural environment microbial evolution as well (Martinez, 2009). Due to their use in livestock farming, antimicrobials may reside in manure applied to soil in agriculture practices. Besides this, AMR bacteria shed by animals may contaminate agricultural areas. In this way, both antimicrobials and AMR bacteria may be run-off from soil and reach surface water, then contributing to contamination of soil, crops and wild animals (Laxminarayan et al., 2013). To their turn, wild animals can enter in close contact with sewage and garbage, thus leading to a wide spread of AMR bacteria and resistance genes, with detrimental consequences for the entire ecosystem (Pesapane et al., 2013).

Bacteria carrying highly transferable resistant genes are of concern, because mobile elements could reach a wide variety of settings in which other bacterial species are present. For example, the use of water from polluted rivers in agriculture and aquaculture increases such risk of AMR bacteria spreading to more than one food chain (EFSA, 2013). One example is given by Salmonella in poultry, E. coli in livestock or K. pneumoniae in dairy cattle. Therefore, when AMR bacteria and their transferable resistant genes are lead to food-producing animals through polluted environment, the risk for potential contamination of their derived products should not be ignored (EFSA, 2013). The studies focused on the detection of CP bacteria from different environmental sources are shown in Table 4.

The detection of carbapenemase-encoding genes in bacteria from livestock environmental samples was reported in pig-fattening farms in Germany, where VIM-1-positive Salmonella Infantis was isolated from boot socks taken outside of the farms and VIM-1-producing E. coli from manure and flies. Indeed, manure and insects can act as vectors of AMR bacteria at farm

<table>
<thead>
<tr>
<th>Bacterial species</th>
<th>Carbapenemase genes</th>
<th>Additional resistant genes</th>
<th>Source</th>
<th>Year</th>
<th>Country</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>S. Infantis</td>
<td>blaQIM1</td>
<td>blaQIM1</td>
<td>Boot socks outside pig farms</td>
<td>2011-2012</td>
<td>Germany</td>
<td>Fischer et al., 2017</td>
</tr>
<tr>
<td>E. coli</td>
<td>blaQIM1</td>
<td>blaQIM1</td>
<td>Manure and flies (Pig farms)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Klebsiella spp.,</td>
<td>blaQIM1</td>
<td>blaQIM1</td>
<td>Hospital sewage WWTP **</td>
<td>2011</td>
<td>Brazil</td>
<td>PicaO et al., 2013</td>
</tr>
<tr>
<td>Enterobacter spp.,</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Citrobacter spp.,</td>
<td></td>
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<td></td>
<td></td>
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<tr>
<td>Serratia spp.,</td>
<td></td>
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<tr>
<td>Raoultella spp.</td>
<td></td>
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<tr>
<td>Aeromonas spp.,</td>
<td></td>
<td></td>
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<td></td>
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</tr>
<tr>
<td>Kluyvera spp.</td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>E. coli</td>
<td>blaQIM1</td>
<td>blaQIM1</td>
<td>WWTP **</td>
<td>2015-2016</td>
<td>Switzerland</td>
<td>Zurfluh et al., 2017</td>
</tr>
<tr>
<td>C. freundii</td>
<td>blaQIM1</td>
<td>blaQIM1</td>
<td></td>
<td></td>
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<tr>
<td>K. pneumoniae</td>
<td>blaQIM1</td>
<td>blaQIM1</td>
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<tr>
<td>E. coli</td>
<td>blaQIM1</td>
<td>blaQIM1</td>
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<td></td>
</tr>
<tr>
<td>P. aeruginosa</td>
<td>blaQIM1</td>
<td>blaQIM1</td>
<td>Hospital sewage water</td>
<td>N.S.</td>
<td>Spain</td>
<td>Scotia et al., 2011</td>
</tr>
<tr>
<td>Brevundimonas</td>
<td>blaQIM1</td>
<td>blaQIM1</td>
<td></td>
<td></td>
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<td></td>
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<tr>
<td>diminuta</td>
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<td>Rhizobium radiobacter</td>
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<tr>
<td>Pseudomonas</td>
<td>blaQIM1</td>
<td>blaQIM1</td>
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</tr>
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<td>montelii</td>
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<tr>
<td>Ochrobactrum</td>
<td>blaQIM1</td>
<td>blaQIM1</td>
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</tr>
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<td>Acinetobacter</td>
<td>blaQIM1</td>
<td>blaQIM1</td>
<td></td>
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<td>johnsonii</td>
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<td></td>
</tr>
<tr>
<td>P. aeruginosa</td>
<td>blaQIM1</td>
<td>blaQIM1</td>
<td>Hospital sewage water</td>
<td>N.S.</td>
<td>Spain</td>
<td>Scotia et al., 2011</td>
</tr>
<tr>
<td>P. fluorescens</td>
<td>blaQIM1</td>
<td>blaQIM1</td>
<td></td>
<td></td>
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<td></td>
</tr>
</tbody>
</table>

**NS.**, not specified. *when reported by the authors. **WWTP: waste water treatment plant.

Table 4. Carbapenemase-producing Enterobacteriaceae and non-Enterobacteriaceae from the environment.
level, maintaining and distributing AMR microorganisms over long periods of time. Furthermore, the detection of both VIM-1 producing \( S. \) Infantis and \( E. \) coli on the same farm was suggestive of the interspecies gene transfer at farm level (Fischer et al., 2017).

Important sources of AMR pathogenic bacteria worldwide are represented by hospitals, which release their sewage drains and wastewater in the environment (Baquero et al., 2008). The spread of CP bacteria from hospitals is a serious menace for the entire ecosystem. Several studies have been performed on aquatic environments, such as hospital sewage, wastewater treatment plants (WWTPs), lakes and rivers and most reports assess that a common way for carbapenemase genes to enter the environment is represented by wastewater. Hospital effluents are normally mixed with urban effluents and treated in WWTPs, to be discharged in the aquatic environment (Verlicchi et al., 2012). In WWTPs, AMR bacteria and their resistant genes can survive (Yang et al., 2016), thus persisting in the environment and spreading back to animals and humans.

WWTPs have been investigated in different countries. In Brazil, the \( \text{bla}_{KPC-2}\) gene was identified in isolates of \( \text{Aeromonas} \) spp. and Enterobacteriaceae belonging to the genera \( \text{Klebsiella} \), \( \text{Enterobacter} \), \( \text{Citrobacter} \), \( \text{Serratia} \), \( \text{Klyuvera} \) and \( \text{Routella} \) present in hospital effluents and WWTPs. The \( \text{bla}_{KPC-2}\) gene of such isolates was successfully transferred \( \text{in vitro} \) to the recipient strain \( \text{E. coli} \) 353, showing that it was located either on conjugative or transference plasmids; this finding supported the hypothesis that the \( \text{bla}_{KPC-2}\) gene could have been transferred to environmental bacteria, such as \( \text{Aeromonas} \), \( \text{Klyuvera} \) and \( \text{Routella} \), from clinical isolates discharged in the hospital sewage (Picão et al., 2013). The acquisition of mobile resistant genes could be promoted by selective pressure exerted by the antimicrobials which are commonly found in hospital sewage (Brown et al., 2006). In Switzerland, in wastewater samples collected before and after the influx of hospital sewage, the increasing of OXA-48 \( E. \) coli, OXA-48 \( C. \) freundii and KPC-2- or OXA-48 \( K. \) pneumoniae downstream the hospital wastewater influx was demonstrated (Zurfluh et al., 2017). In Austria, KPC-2 \( K. \) pneumoniae, OXA-48 \( E. \) coli and OXA-48 \( K. \) pneumoniae strains were detected from a WWTP collecting from both domestic and hospital effluents, showing that CP Enterobacteriaceae could escape from clinical settings to be released into the aquatic environment (Galler et al., 2014). In China, KPC-2 producing bacteria belonging to the genera \( \text{Klebsiella} \), \( \text{Enterococcus} \), \( \text{Escherichia} \), \( \text{Shigella} \), \( \text{Wautersiella} \), \( \text{Acinetobacter} \) and \( \text{Stenotrophomonas} \) were recovered from WWTP effluent samples. Chlorination treatment was not effective, being different genes \( \text{bla}_{KPC-2}, \text{bla}_{GES-1} \) and \( \text{bla}_{(CIP)} \) detected in large amounts in all sections of the plant. In this study, horizontal transfer of \( \text{bla}_{KPC-2}\) gene was demonstrated in \( \text{Paenibacillus} \) spp., an environmental microorganism which was never found before to harbor carbapenemase genes (Yang et al., 2016). In Tunisia, \( \text{bla}_{KPC}, \text{bla}_{NDM} \) and \( \text{bla}_{OXA-48}\) genes were detected in high concentrations from hospital wastewater samples, demonstrating the widespread of CP bacteria in hospitals as well as their potential source of environment pollution (Nasri et al., 2017). In Spain, VIM-13-producing \( P. \) aeruginosa was found in the sewage water of a hospital, together with isolates of VIM-13-producing \( \text{Brevundimonas} \) diminuta, \( \text{Rhizobium} \) radiobacter, \( \text{Pseudomonas} \) monteiili, \( \text{Ochrobactrum} \) anthropic and \( \text{Acinetobacter} \) johnsonii. These findings suggested that the environmental microbiota could represent a reservoir of resistance genes, probably acquired by genetic transfer from pathogens like \( P. \) aeruginosa (Scotta et al., 2011).

Other studies support the hypothesis that “sewage habitats” may represent a niche for AMR microorganisms, enhancing the potential transfer of genetic determinants among different bacterial species (Scotta et al., 2011) and bacterial dissemination to rivers (Novais et al., 2005) and coastal waters (Quinteira and Peixe, 2006). In Portugal, \( \text{bla}_{VIM}\)-harboring \( P. \) aeruginosa strains were isolated from a river and a hospital sewage downstream. The \( \text{bla}_{VIM}\) gene was located on a class 1 integron (Quinteira and Peixe, 2006). In Tunisia, \( \text{bla}_{OXA-3}, \text{bla}_{OXA-19}\) and \( \text{bla}_{OXA-11}\) \( K. \) pneumoniae isolates were identified from polluted rivers and class 1 integrons were considered the vectors for transmission of \( \text{bla}_{NDM}\) genes among the bacteria (Chouchani et al., 2013). Even seepage water (for example, water pools in streets) might be a source of CP bacteria, as \( \text{bla}_{NDM}\)-harboring \( K. \) pneumoniae, \( E. \) coli, \( C. \) freundii, \( S. \) boydii, \( V. \) cholerae, \( A. \) caevae, \( P. \) aeruginosa, \( P. \) putida, \( S. \) maltophilia and other species in India (Walsh et al., 2011). From the Seine River, in Paris (France), \( \text{bla}_{OXA-23}\)-harboring \( A. \) baumannii was recovered (Girlich et al., 2010a), together with \( \text{bla}_{BIC-1}\)-harboring \( P. \) fluorescens (Girlich et al., 2010b). The enzyme BIC-1, a novel class A chromosome-encoded \( \beta\)-lactamase, hydrolyzes penicillins, carbapenems and cephalosporins, except ceftazidime and monobactams (Girlich et al., 2010b); it shares high identity with SFC-1 from \( S. \) fonticola (Henriques et al., 2004) and the plasmid-encoded KPC-2 from \( \text{Klebsiella} \) spp. (Yigit et al., 2003).

At last, the role of natural environment in maintaining and spreading CP microorganisms can be suggested by a study conducted in Switzerland in ready-to-eat vegetables, where OXA-181-producing \( K. \) variicola was identified from a coriander mix sample imported from Thailand/Vietnam (Zurfluh et al., 2015). This finding not only sheds light on the potential role of vegetables in transmitting CP bacteria to the consumers, but suggests that such a role is very likely attributable to environmental contamination. Nevertheless, contamination of vegetables does not necessary derive from the original harvesting environment, and might originate from the different processing and commercial steps they are subjected as well.

In conclusion, the environment can host CP bacteria both from human and animal sources, maintaining and distributing AMR microorganisms and their genetic determinants to different settings. As recently suggested (Scotta et al., 2011), the environment microbiota can support the genetic transfer among bacteria, especially mediated by class 1 integrons, from pathogenic to environmental microorganisms. The role of the natural environment as reservoir of CP bacteria is probably more important than what we actually hypothesized. In Europe as well as in other parts of the world, more efforts should be warranted especially in countries where human pathogenic CP microorganisms are endemic.

Wildlife and carbapenemase-producing bacteria

Actually, the potential role of wildlife in the maintaining and dissemination of resistance genes and AMR bacteria in the natural environment and livestock animals is far to be fully understood. AMR bacteria can colonize wild animals following contact with sewage, human waste or animal manure, with serious issues for public and animal health and ecosystem functions (Pesapane et al., 2013). The intake of water polluted with faeces or human waste seems to be the most important route for wild birds to acquire AMR bacteria of human origin (Guenther et al., 2011). In addition, the intake of polluted water with faeces of live-stock animals could have the same importance for wild birds and wild animals to acquire AMR bacteria of farm origin. At present, only four studies have detected CP
bacteria in wild animals (Table 3). The first report originated from Germany, where a strain of blaNDM-1 Salmonella Corvallis belonging to ST1541 was isolated from black kites (Milvus migrans). The blaNDM-1 gene was located on the ~180 kb IncA/C conjugative plasmid pRHI-1738 (Fischer et al., 2013b). Since the IncA/C plasmids are among the most common plasmids associated with the blaNDM-1 gene in humans (Carattoli, 2013) and S. Corvallis was more common in South-East Asia, North Africa and Nigeria rather than in Europe, Fischer and colleagues (2013b) supposed that the isolate might have originated from non-European countries and transferred to Germany through the black kite migratory route. The sequence of the plasmid pRHI-1738 confirmed this hypothesis (Villa et al., 2015) and the additional presence of the resistant gene for fosfomycin (fosA3), which is common in Asia, strongly supported for the Asiatic origin of the blaNDM-1 fosA3-harboring S. Corvallis (Qin et al., 2014).

In France, VIM-1-producing E. coli in yellow-legged gulls (Larus michaellis) has been recently reported (Vittecoq et al., 2017). In the geographical area of the study, yellow-legged gulls live in close contact with humans and all the CP isolates from gulls were closely related to carbapenem-sensitive E. coli of human patients hospitalized in the area. Since the blaVIM-1 gene is uncommon in France, but can be frequently found in human isolates from Greece, Italy and Spain (Cantón et al., 2012; Mathlouthi et al., 2017) the authors warned over the potential role of wild birds as carriers of carbapenem-resistance bacteria and their encoding genes from endemic countries (Vittecoq et al., 2017).

In south-east Australia, large-scale transmission of IMP-producing bacteria, into wild birds was reported. E. coli strains harboring blaIMP-4 or blaIMP-26 genes, K. pneumoniae harboring blaIMP-4 or blaIMP-26 genes, Citrobacter freundii harboring blaIMP-26 gene and Enterobacter aerogenes and Proteus mirabilis harboring blaIMP-4 gene were isolated from silver gulls (Chroicocephalus novaehollandiae). The blaIMP-4 gene was carried by various conjugal plasmids, mostly IncHI2-N plasmid type, and was associated with a class 1 integron (Dolejska et al., 2016). Interestingly, blaIMP-4 is the most commonly detected gene among CPE affecting human patients in Australia (Sijabat et al., 2015; Espedido et al., 2008). PCR mapping revealed the blaIMP-4-qacG-aacA4-catB3 cassette array in 65% of E. coli isolates and 80% of non-E. coli isolates of gull origin (Dolejska et al., 2016). The same cassette array was found in Enterobacteriaceae from humans in other Australian towns (i.e. Sydney and Melbourne) (Espedido et al., 2008), strongly suggesting the human origin of the IMP-producing E. coli and the other Enterobacteriaceae detected in gulls. The hypothesis was also supported by the feeding habits of the gulls on waste depots, where garbage and sewage were almost exclusively related to human activities and the risk of transmission of bacteria from the human compartment to the wild one was high (Dolejska et al., 2016).

Thus, even if carbapenem-resistance in bacteria from wild animals is rarely reported, the emergence of NDM-1 and IMP carbapenemases in wild birds should not be ignored because of their migratory habits and the consequent ability of long-distance transportation of AMR resistant bacteria and their related genes.

Regarding wild mammals, the first report of OXA-48 producing Enterobacteriaceae originated from Africa. In Algeria, faecal samples from 168 wild boars (Sus scrofa) and 212 barbary macaques (Macaca sylvanus) were collected between 2014 and 2016 and tested for carbapenem-resistant genes. Two blaOXA-48 carrying E. coli ST635 and one blaOXA-48 carrying K. pneumoniae ST13 were isolated from wild boars, thus confirming dissemination of CP bacteria to wild animals (Bachiri et al., 2018) in a country where occurrence of carbapenem-resistant bacteria in humans (Sassi et al., 2014), pets (Yousfi et al., 2015) and livestock animals (Yaici et al., 2016) had been previously reported.

Future studies

This review has shed light on the main scientific gaps which require future studies, summarized as follows: i) CP bacteria occurrence in food-producing animals, especially pigs, cattle and poultry, has not been sufficiently investigated in the countries characterized by high prevalence of CP bacterial infections in humans; ii) the correlation between CP bacteria occurrence and antimicrobial use at farm should be better investigated, with special regard to third- and fourth-generation cephalosporins use; iii) the observed co-resistance against cephalosporins and carbapenems needs to be investigated by genomic sequencing of the isolates; iv) comparative genomic studies of CP isolates from humans, animals and the environment should be encouraged to investigate the likely transmission pathways between the different compartments.

Conclusions

Several factors may contribute to select AMR bacteria in a host, due to their opportunity to become more prevalent as the result of killing the sensitive bacterial population by antimicrobials to which they exhibit reduced susceptibility. As a consequence, the AMR bacteria can become dominant among the previously resident bacterial population (Baquero et al., 2011). Besides this, for many classes of antibiotics, selection of plasmids carrying resistance determinants occurs especially at antimicrobial concentrations far below the MIC value of a susceptible strain. Low antimicrobial concentrations kill only a fraction of the cells, whereas the rest of the bacterial population may evolve to reduced susceptibility or resistance. Accordingly, even the low levels of antibiotics often present in treated animals and humans as well as in polluted natural environments could enhance selection and enrichment of bacteria with transferable resistance genes and thereby contribute to the emergence, maintenance and transmission of AMR bacteria (Guerra et al., 2014; Gullberg et al., 2014).

Among AMR bacteria, CP microorganisms are of particular concern for public health because carbapenemases are considered the “last-line defence” drugs against human infections by multidrug resistant Gram-negative bacteria (Zhanel et al., 2007; Nordmann et al., 2011; Patel and Bonomo, 2013). Despite the use of carbapenems in fly-producing animals is banned in Europe and other countries (WHO, 2015), detection of CP microorganisms in livestock has been reported in EU as well as non-EU countries. Selection pressure exerted by β-lactams antimicrobial treatments in farm animals, as well as CP bacteria transmission from human sources, are considered responsible for the occurrence of CP microorganisms in livestock. Likely, meat and milk might become a source of CP bacteria for the consumers, with the worst scenario to be displayed in countries with high prevalence of CP bacterial infections in humans.

In this context, the role of hospital sewage and WWTPs in the environmental distribution of human CP bacteria has been effectively assessed. Furthermore, human activities on natural habitats, as the use of manure to amend soil in agricultural practices, strongly contribute to the diffusion and maintenance of resistance bacteria and their transferable genetic elements in the terrestrial and aquatic environments. In this scenario, wild animals, and especially migratory birds, may amplify both mainte-
formance and long-distance distribution of CP microorganisms.

Recently, pollution of the aquatic environment by AMR bacteria, and especially CP bacteria, has attracted the greatest attention all over the world. Accordingly, even the fishery chain might be responsible for transmission of CP bacteria and their transferable genetic determinants to consumers.

According to the epidemiology of CP bacteria, monitoring studies in food-producing animals, environment and wildlife should be warranted especially in the geographical areas where prevalence of CP invasive bacteria in humans is high. For instance, in Europe, southern and eastern countries reported the highest prevalence of carbapenem-resistant bacteria in human settings. In decreasing order, CP bacterial infections are mostly reported by Greece, Italy, Romania, Cyprus and Bulgaria for K. pneumoniae; by Romania, Latvia, Slovakia, Greece and Hungary for P. aeruginosa; by Croatia, Greece, Lithuania, Romania and Bulgaria for Acinetobacter spp. (ECDC, 2018).

Several studies agree that antibiotic resistance plasmons and other transferable elements are circulating among livestock and wild animals worldwide and across vertebrate species barriers (Chang et al., 2015). Although the overall prevalence of CP microorganisms in food-producing animals and wildlife appears to be low, CP bacteria transmission from food-producing animals to their derived products could be a threat to consumers, thus promoting mobile carbapenemase gene pools in human enteric flora and supporting transmission of resistant determinants between commensal and pathogenic microorganisms with unknown, but potentially severe, consequences for human health.

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