ANTIBIOTIC RESISTANCE AMONG ENTERIC BACTERIA AND THEIR HEALTH IMPLICATION

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Abstract

Today antimicrobial agent resistance is an emerging global concern to both public and veterinary health. The use of antibacterial drugs for prophylactic or therapeutic purposes in humans and for veterinary and agricultural purposes has provided selective pressure favoring the survival and spread of resistant organisms. However, resistant bacteria may transfer their resistance to previously non-resistant pathogenic bacteria or directly infect humans with bacterial diseases that cannot be treated by conventional antimicrobial therapies. The potential for antibiotic exposure and resistance development in human and animal gastrointestinal tracts, coupled with relatively great abundance in waters contaminated with human and animal waste, makes the fecal coliform bacteria a logical fecal group for studies of antibiotic resistance and transfer in aquatic environments. The main bacteria present in human and animal feces discussed which indicators of fecal pollution should be used in current drinking water microbiological analysis. This review mainly focused on antibiotic resistance of environmental isolates is imperative to explore the antibiotic pressure in the environment. In addition methods to reduce bacteria resistant load in wastewaters and the amount of antimicrobial agents is originated in most cases of hospitals and farms that optimization the disinfection procedures and management of wastewater.

Key Words: Antibacterial drugs, Bacterial disease, contaminated water, feces, fecal coliform bacteria, Resistance, wastes water

I. INTRODUCTION

As it is well known that antimicrobial resistance is a global problem in human and veterinary medicine. Antibiotic resistance in bacteria provides one of the well-documented examples of an evolutionary response to selection in natural populations. The selective agent can be easily identified, changes in phenotype have occurred in a human generation, and the mode of transmission of resistance determinants can be deduced [1-3]. The antimicrobial agents used in animal care are also significant, not only in increasing the resistance in animal pathogens, but also in bacteria transmitted from animals to humans. Most studies show that not only the level of resistance of pathogenic bacteria, but also of commensal bacteria increases. It is generally accepted that the main risk factor for increase in the antibiotic resistance is extensive use of antibiotics. This has lead to the emergence and dissemination of resistant bacteria and resistance genes in animals and humans. Dramatically rapid increases in antibiotic resistance have been observed in a host of phylogenetically diverse taxa and the process has occurred repeatedly within taxa as each is challenged with new antibiotics [1, 4, 5]. Antibiotic selection pressure can lead to a rapid reduction in the initial fitness costs that were obtain as a pleiotropic effect of resistance. Additional consequences of the rapid evolution of resistance may depend upon population structure. Bacterial population structures range from distinguishable clonal to non-clonal, depending on the relative roles of horizontal and vertical transmission of genetic information within a species [4, 6]. Antibiotic
resistance determinants can spread via either clonal elaboration or horizontal transfer, the latter mediated by conjugative plasmids, phage vectors, and natural transformation systems. In contrast, the spread of resistance determinants via promiscuous horizontal exchange can be expected to both maintain variation and show an absence of linkage disequilibrium between the selected and neutral markers. Studies based on samples from a single time point often rely upon variation in resistance genes and linkage disequilibrium to distinguish vertical from horizontal transmission. The subsequent intraspecific spread of chromosomal resistance genes was most likely mediated by a combination of clonal elaboration, as evidenced by linkage disequilibrium between resistance genes and housekeeping genes, and horizontal gene transfer, deduced from the incongruence of dendrograms portrayaling patterns of variation within the two types of loci [3, 7]. Investigation of the prevalence of resistance of certain indicator bacteria like E. coli and enterococci in the intestinal tract of different populations of animals and humans makes it workable to compare the prevalence of resistance in different populations. Because of the inavoidable high usage of antibiotics in hospitals, selection and diffusion of resistant clones and resistance genes is high in hospitals. Therefore, healthy individuals in the community outside hospitals hold not only a reservoir of resistant bacteria and resistant genes, but are considered to be a suitable population to study the possibility of transfer of resistant bacteria or resistance genes from animals to humans [1]

II. DETECTION OF MICROORGANISM

The detection and reckoning of microorganism trust on cultural methods. In these methods, the microorganism is grown on either a solid (agar) or liquid (broth) medium, which supplies the nutritional requirements of the organisms or in the case of obligate parasites such as viruses, the organism is grown in a culture of host cells. Once a microorganism has been grown and isolated as a pure culture, identification of the organism is based on biochemical, immunological (serological), and genetic characteristics of the isolate. In many illustrations, particularly for such well studied organisms as the coliforms, specific compounds are obtain into the primary media, which allow for selection and differentiation of the organisms of interest. For example, Endo agar is routinely used for the reckoning of coliforms and otherenteric organisms. This agar has sodium sulfite and basic fuchsin to inhibit the growth of Gram-positive bacteria. In addition, lactose is present as a primary substrate for bacterial nutrition. Metabolism of lactose with the formation of acid and gas, a hallmark characteristic of the coliform group, is detected by a color change due to the reaction between acetaldehyde (an intermediate product of lactose fermentation) and the sodium sulfite. Within the past several decades alternative, noncultural methods for the detection of microorganisms have been developed and are now widely used. These methods are particularly useful for the detection of microorganism for which no selective media has yet been developed. These methods include the use of specific staining procedures, usually based on serological properties of the organism (immunofluorescence), and molecular methods for the detection and characterization of specific sequences within the DNA or RNA of the organism. Immunofluorescent detection of microorganisms depends on the ability of antibody molecules to recognize and react with specific three-dimensional regions (epitopes) on the surface of microbial cells. Antibodies can be labelled with fluorescent dyes. Specific-microorganisms stained with immunofluorescent dyes can be enumerated by direct counts under an epifluorescentmicroscope. Detection of nucleic acid sequences unique to a particular organism involve several distinguishable procedures. Nucleic acid probes containing nucleotide sequences complementary to a unique sequence of a specific microorganism can be coupled with a variety of reporter molecules (fluorescent dyes and radioisotopes). When mixed with a solution of DNA elicit from an environmental sample, the probe will only bind to those specific target sequences that are complementary to it. Thus, the presence of specific microbial nucleic acid, and presumably then specific microorganisms, can be determined. Frequently, the amounts of specific nucleic acids present in environmental samples are too low to be detected directly by gene probes. In this case, there is a need to increase, or amplify, the specific
sequences to detectable levels. This is accomplished through the use of the polymerase chain reaction (PCR).

III. INDICATOR BACTERIA

Indicator bacteria belonging to the normal intestinal flora of humans and animals. These bacteria not only constitute an tremendous reservoir of resistance genes for (potentially) pathogenic bacteria, but also the level of resistance in the endogenous flora is considered to be a good indicator for the selection pressure exerted by antibiotic use in that population [8] and for the resistance problems to be expected in pathogens [9]. Indicator organisms that can be transmitted by a waterborne route, (e.g., thermotolerant coliforms) are used widely as deputy for the detection of pathogens.[10]. Compared two media, mFC and mTEC, for the reckoning of fecal coliforms in tidal creeks. Counts by mTEC were systematically higher than mFC counts at all salinity ranges. In addition, a significant number of false positives were associated with using mFC in middle and high salinity areas. Lifshitz and Joshi found the ColiPlate (CP) kit gave estimates of E. coli that were 20% higher than standard membrane filtration when the two methods were compared for testing water samples [11]. The difference increased when samples were impaled with injured cells. They concluded that the CP kit is a more reliable method for use with samples having high levels of injured or weakened cells. m-ColiBlue24 (m-CB) was compared to m-Endo medium and an International Organization for Standardization (ISO) standard conform medium, lactose agar with Tergitol 7, for the analysis of indicator organisms in bottled water. Pruss reviewed studies on uncontrolled waters, such as seas, lakes, and rivers, to evaluate the health risks caused by poor microbiological quality of recreational water. Most studies reported a dose-related increase of health risk in swimmers with an increase in the indicator bacteria count in recreational waters. The indicator microorganisms that correlated best with health out come were Enterococci/Fecal streptococci for both marine and freshwater and E. coli for freshwater [10].

(1) Fecal Indicator Bacteria

In 1892, Schardinger proposed that since-Bacterium coli was a characteristic component of the fecal flora, its presence in water could be taken as an indication of the presence of fecal pollution and therefore of the potential presence of enteric pathogens [12]. Various classification schemes for coliforms have emerged. The earliest were those of MacConkey in 1909, which recognized 128 different coliform types, while Bergey and Deehan in 1908 identified 256. By the early 1920s, differentiation of coliforms had come to a series of correlations that suggested that indole production, gelatin liquefaction, sucrose fermentation and the Voges-Proskauer reaction were among the more important tests for determining fecal contamination. These developments culminated in the IMViC (Indole, Methyl Red, Voges-Proskauer and Citrate) tests for the differentiation of so-called fecal coliforms and intermediates [13]. Traditional tests for total and fecal coliforms are carried out either by the multiple-tube fermentation technique or by filtration through membrane. The multiple-tube fermentation technique is used for medium or highly contaminated waters, and the filtration through membrane for low or very low contaminated waters. Filtration through membrane is a very sensitive technique since can detect one (culturable) cell in 500 or even 1,000 mL of water. However, both methods take several days to complete and do not detect viable but non-culturable bacteria [14]. These limitations stimulate the discovery of alternative methods, faster and, if possible, less prone to false negative results such as those caused by the viable but non-culturable bacteria. Fecal Indicator bacteria are generally three types that found on the basis of different test and describe below following.

3.1.1 Total Coliforms

Total coliforms are Gram-negative, oxidase-negative, non-sporeforming rods, which ferment lactose with gas production at 35-37 °C, after 48h, in a medium with bile salts and detergents [15]. When the test of coliforms is carried out with environmental waters, several species of the four Enterobacteriaceae genera Escherichia, Klebsiella, Enterobacter and Citrobacter give positive
results and therefore are coliforms according to this definition. However, the environmental significance of these four genera is very disparate as discussed in the present text. Therefore, total coliform counts are not necessarily a measure of fecal pollution and indeed can have no relation with this cause [16]. The detection of β-D-galactosidase activity (at 37 °C) is usually a good marker for total coliforms in environmental waters, since most of these bacteria display this enzymatic activity [2, 9]. Most *Escherichia*, *Citrobacter*, *Enterobacter*, *Klebsiella* and *Raoultella* strains have galactosidase. *Hafnia*, *Serratia* and *Yersinia* also possess this enzymatic activity. Most *Proteus*, *Salmonella* and *Edwardsiella* strains do not display β-galactosidase [17, 18]. β-galactosidase cleaves lactose in glucose and galactose, and can be detected by using colored or fluorescent markers that change color after enzyme action. In environmental waters, the presence of *Aeromonas* or *Vibrio cholerae* can be a source of false positives in the β-D-galactosidase assay, since these bacteria have galactosidase, but are not coliforms. Additionally, in particular environments, such as estuaries, β-galactosidase activity can overestimate total coliform count due to UV-stimulated enzymatic activity in certain bacteria such as *E. coli*.

3.1.2 Fecal coliforms

Fecal coliforms (or thermotolerant coliforms) are traditionally defined as coliforms that ferment lactose at 44.5 °C in a medium with bile salt[15]. The range of species detected by the experimental procedure is much lower than that of total coliforms. With environmental polluted waters, only *E. coli*, and *Klebsiella* and *Klebsella* strains gave positive results in the test [19]. The detection of β-D-glucuronidase activity (at 44.5 °C) is, generally, a good marker for fecal coliforms in environmental polluted waters and very specific for *E. coli* [20]. In Gram-negative bacteria, this enzymatic activity if found in most *E. coli* strains and in some *Salmonella* and *Shigella* strains [21]. *Aeromonas*, *Citrobacter*, *Enterobacter*, non-coli *Escherichia*, *Hafnia*, *Klebsiella*, *Proteus*, *Serratia*, *Vibrio*, *Yersinia*, and most *Salmonella* strains do not display β-glucuronidase activity [20]. β-D-glucuronidase activity can be detected by using colored or fluorescent markers that change color after enzyme action. The presence of this enzyme in some strains of *Bacteroides*, *Flavobacterium*, *Staphylococcus*, *Streptococcus*, in anaerobic *Corynebacterium* and *Clostridium*, has also been reported [22]. β-D-glucuronidase activity in fecal bacteria other then *E. coli* (*Bacteroides*, *Bifidobacteria*, *Clostridia*, *Enterococci* and *Lactobacillus*) is very limited [23].

3.1.3 Streptococci and Enterococci

Fecal streptococci also belong to the traditional indicators of fecal pollution. Fecal streptococci are Gram-positive, catalase-negative, non-sporeforming cocci that grows at 35 °C in a medium containing bile salts and sodium azide[15] Azide is a strong inhibitor of the respiratory chain. Since streptococci are one of the very few bacteria that have no respiratory chain, the test is very specific for this group, and false positives are rarely found [24]. Fecal enterococci (*E. faecalis*, *E. faecium*, *E. avium* and *E. gallinarum*) are fecal streptococci that grow in the presence of 6.5% NaCl at 45 °C. Selective media use these particular characteristics in order to separate enterococci from the other streptococci. Several studies have reported on the microbiological composition of human and animal (cattle, chicken, deer, dog, fowl, goose, and swine) feces. *E. faecalis* and *E. faecium* were present in human and animal feces. However, whereas human feces almost have only these two enterococci, in the animals others species co-occur, like *E. avium*, *E. cecorum*, *E. durans*, *E. gallinarum* and *E. Hirae* [25].

IV. ANTIMICROBIAL RESISTANCE

Many retrospective and prospective studies have been performed to study the Egression and selection of resistance in bacteria by antibiotic usage. Despite large differences in methodology, most results show that after the introduction of an antibiotic in veterinary practise, the resistance in pathogenic bacteria and the faecal flora increases, as in human medicine. Some bacteria, most *Enterobacteriaceae*, *Staphylococci* and *Pasteurella spp.* become more readily resistant to certain antibiotics than others like *Clostridium sps.* and *streptococci* which are still fully susceptible to
penicillinG. The literature on resistance against APE is very limited as most of these molecules are not used for therapy and therefore, susceptibility testing is not performed regularly. Linton et al. found a significant increase in the prevalence of resistance against tylosin and bacitracin in faecal enterococci of fed these molecules [26]. In this study, virginiamycin usage did not result in an increase in resistance. Ohmae et al. noticed an increase of resistance against carbadox in faecal *E. coli* isolates of pigs after its introduction as APE [27]. All resistant isolates from six farms that fed carbadox continuously to pigs either as APE or for prevention of swine dysentery carried the same transferable plasmid consulting carbadox resistance. Carbadox is not used in poultry and no carbadox resistance was found in *E. coli* isolates from poultry in the same region. Mills and Kelly [28] also reported an increase in resistance in *E. coli* isolates from 37 to 61% after the introduction of carbadox. Carbadox, however, was not only used as an APE, but also for prevention of swine dysentery and therapy for salmonellosis. Interest in the selection of resistance by APE increased after the Egression of vancomycin resistant enterococci (VRE) in human infections. It was soon recognised that avoparcin was until recently commonly used as APE in most EU-member states, selects for VRE in the intestinal flora of animals. In countries where avoparcin was used as APE, VRE was not only found in food animals fed with avoparcin, but also in the faecal flora of healthy humans and pet animals [29, 30]. Also resistance against MLS-antibiotics like erythromycin and quinupristin-dalfopristin is quite common in enterococci from animals fed with related antibiotics as APE like tylosin (a macrolide) or virginiamycin[30]. According to WHO the resistance to antibiotics is an ability of bacterial population to survive the effect of inhibitory concentration of antimicrobial agents. Antimicrobial resistance in bacteria may emerge by several pathways. Some bacterial species are normally and inherently resistant to certain antibiotics, whereas other are sensitive. Sensitivity has 3 requirements: a target for reaction, a mechanism for transport into the cell before the antibiotic action takes place and absence of enzymes that could inactivate or modify the antibiotic. A change in any of these prerequisites could render an antibiotic-sensitive bacterium resistant to the drug [31]. Water bacteria might be endemic to aquatic environments or exogenous, transiently and occasionally present in the water as a result of shedding from animal, vegetal, or soil surfaces. The study of antibiotic resistance in endemic water organisms is important, as it might indicate the extent of modification of water ecosystems by human action. Aeromonas strains from Portuguese estuarine water carry less frequently beta-lactamase genes than Enterobacteriaceae[32]. In water reservoirs about half of Aeromonas strains might present multiple antibiotic resistance [33]. Resistance profiles of aquatic pseudomonads depend on the species composition, but also from the site in which they were isolated, being more antibiotic-resistant along shorelines and in sheltered bays than in the open water, indicating the influence of nonaquatic organisms or pollutants. Nevertheless, such influences can be found in the more remote water environments; psychrotrophic bacteria from Antarctic show various degrees of resistance to industrial antibiotics and metals [34].

The connexion of antibiotic-resistance and resistance to heavy metals is very frequent in the same organism (also in the same plasmid, transposon, or integron) so that industrial pollution probably selects for antibiotic-resistance and vice versa [35]. Indeed metal contamination represents a long-standing, widespread, and fractious selection pressure for multiresistant organisms. For the nonaquatic organisms, obviously the density of antibiotic-resistance organisms and antibiotic-resistance genes in fresh water varies with the proximity to areas with increased antibiotic expenditure, metal pollution, and between seasons, being more frequently found in rainy seasons [1]. Very little work has been done to illuminate the role of bacterial biofilms in water environments and its role in antibiotic resistance. Phenotypic antibiotic resistance in bacterial biofilms might indeed protect the water environment from selective events caused by the antibiotic release, which probably are acting more effectively on planktonic bacteria.

### 4.1 Mechanism of antibiotic resistance in bacteria

The development of antibiotic resistance is the ability of infectious organisms to adapt quickly to new environmental conditions. Bacteria are single-celled organisms that, compared with higher life forms, have small numbers of genes. Therefore, even a single random genetic mutation
can greatly affect their ability to cause disease and because most microbes reproduce by dividing every few hours, bacteria can evolve rapidly. A mutation that helps a microbe surviving to an antibiotic exposure will quickly become dominant throughout the microbial population. Microbes also often evolve resistance genes from each other through horizontal gene transfer mechanism which might enable them to be a multiple antibiotic resistant strain. It is also noted that the specificity of the interactions between antibiotics and various protein sequences within a bacterium resultse in significantly high ratio of mutations in its genome which leads to antibiotic resistance. There is also a relatively high possibility that a particular mutation in a certain target sequence will result in antibiotic resistance. Antibiotics generally target a variety of essential bacterial functions. For instance, the β-lactam antibiotics and vancomycin interrupte cell wall synthesis of pathogens, whereas macrolides and tetracyclines disrupt the protein synthesis at ribosomal level. Bacteria may develop their antibiotic properties by a variety of mechanisms. One mechanism of resistance is by degrading the antibiotic in a step by step process. This degradation starts when bacterial β-lactamases hydrolyzes the β-lactam ring thus rendering these antibiotics ineffective. A secondary resistance mechanism is then triggered when the antibiotic target is altered. As the next step, bacteria may block the entry of antibiotic to the site of action, resulting in decreased absorption, which in turn results in bacteria with decreased sensitivity to vancomycin due to thicker cell walls. Finally, bacteria may develop efflux pumps that actively pump antibiotics out of the cell so that they do not reach their target[1].

4.1.1Horizontal gene transfer of antibiotic resistance in water environments

Unlike eukaryotes, prokaryotes do not reproduce sexually, nor do they undergo meiosis. Horizontal gene transfer occur in prokaryotes have evolved three different mechanism conjugation, transformation and transduction for creating recombination. Horizontal gene transfer occurs primarily between members of same species. Horizontal gene transfer will become an important in evolution of many species. Antimicrobial resistance in bacteria associated with different ecological niches will be a global concern. Theegression of antimicrobial resistant strains of pathogenic bacteria will become a great threat to the public health. The detection of rising trends in antimicrobial resistance of bacterial strains facilitates implementation of effective control measures. The antibiotic susceptibility testing contributes directly to patient care, and of antimicrobial drug resistance. However, in our region, the study of antibiotic resistance of bacteria from environment like soil, water or from fish is bare. Therefore, study pertaining to antibiotic resistance of environmental isolates is imperative to explore the antibiotic pressure in the environment. Methods to reduce bacteria resistant load in waste waters and the amount of antimicrobial agents is originated in most cases of hospitals and farms that optimization the disinfection procedures and management of waste water.

Estuarine water-borne Aeromonas strains carry almost as frequently as Enterobacteriaceae class 1 integron platforms carrying antibiotic-resistance genes [32]. Exclusively environmentally based organisms, as Delftia, also harbor class 3 integrons. The continuity of such genetic structures cannot probably be explained entirely by antibiotic selection, suggesting that activities resulting in antibiotic resistance might have other physiological roles or that they are placed in multifunctional plasmids. The most frequent gene cassette found involves aminoglycoside-resistance genes, rarely under positive selection in our days, and there is a distrust that some other resistance genes, as integronsul genes, might provide benefits for the bacteria, unrelated with resistance. However, some of these mobile gene cassettes in Aeromonas might involve important mechanisms of resistance, as Qnr, involved in fluoroquinolone resistance, which might behorizontally propagated by IncU-type plasmids [36]. Certainly the dense bacterial populations in sewage treatment plants favor genetic exchange among bacterial populations and communities, integrons predating transposons and plasmid diffusion. Multiresistance plasmids of broad host-range are systematically recovered in sewage [37]. Interestingly, antibiotic-resistance genes from muck influence the lagoons and groundwater gene pool, but this pool also contains antibiotic-resistance genes from endemic bacteria. Aeromonas from aquaculture water systems (fish, eel farming) are particularly resistant to
antibiotics [38] (Penders and Stobbering, found that frequently contain plasmids and integrons with multiple genes for antibiotic resistance [39]. Jacobs and Chenia and the connexion with heavy-metal resistance is not uncommon [35]. Water originated in transgenic plant fields may constitute a matter of concern, but no significant differences have been found in bacterial antibiotic-resistance levels between transgenic and nontransgenic corn fields [40].

V. IMPACT OF ANTIBIOTIC RESISTANCE ON HUMAN HEALTH

Bacteria and other microorganisms that often cause infections are known to be remarkably resilient and have the ability to develop ways for surviving drugs that are meant to kill or weaken them. Recent scientific evidence suggests that during the last decade, antibiotic resistance by various mechanisms has increased worldwide in bacterial pathogens leading to treatment failures in human and animal infections. However, the resistance against different types of biocides (including disinfectants, antiseptics, preservatives, sterilants) has been studied and characterized [41,42]. Biocides and antibiotics may share some common behaviour and properties in their respective activity and in the resistance mechanisms developed by bacteria [43, 44]. Although antibiotic usage has clearly benefited the animal industry and helped providing affordable animal protein to the growing human population, the use of antibiotics in food production has also contributed to the Egression and spread of antibiotic multiple resistances (AMR). Along with antibiotics used for human medicine, the use of antibiotics for animal treatment, prophylaxis and growth promotion exerts an immeasurable amount of selective pressure toward the egression and multiplication of resistant bacterial strains. Animals can serve as mediators, reservoirs and disseminators of resistant bacterial strains and/or AMR genes. Consequently, imprudent use of antimicrobials in animals may eventually result in increased human morbidity, increased human mortality, reduced efficacy of related antibiotics used for human medicine, increased healthcare costs, increased potential for carriage and diffusion of pathogens within human populations and facilitated Egression of resistant human pathogens. The patients infected with pansusceptible Salmonella typhimurium are 2.3 times more likely to die within 2 years after infection than persons in the general Danish population, and that patient infected with strains resistant to ampicillin, chloramphenicol, streptomycin, suldonamide and tetracycline are 4.8 times more likely to die within 2 years. Furthermore, they have established that quinolone resistance in this organism is associated with a mortality rate 10.3 times higher than the general population. It has been well documented that antimicrobial resistance due to a particular antibiotic used in food animals may result in reduced efficacy of most or all members of that same antibiotic class, some of which may be extremely important for human medicine. The current pharmaceutical era faces multi resistant infectious disease organisms that are difficult and, sometimes, impossible to treat successfully. When there is an increase in numbers of bacteria that are resistant to antibiotics, it will be more difficult and more expensive to treat human bacterial infections (Fig 1)[32].

Figure 1. The Human Health Impact of Antimicrobial Resistance in Animal Populations (Adapted from Jalal et al 2012)
VI. EMERGING WATERBORNE BACTERIAL PATHOGENS

The rising pathogenic bacteria of concern outlined here have the potential to be spread through drinking water, but they do not correlate with the presence of E. coli or with other commonly used drinking water quality indicators, such as coliform bacteria. In most cases, there are no satisfactory microbiological indicators of their presence. More studies are needed in order to understand the real significance and dimension of the diseases caused by water contaminated with these bacteria, and the ecology of these pathogens [45].

6.1 Escherichia

*Escherichia*, a member of Enterobacteriaceae, are oxidase-negative catalase-positive straight rods that ferment lactose. Cells are positive in the Methyl-Red test, but negative in the Voges-Proskauer assay. Cells do not use citrate, do not produce H₂S or lipase, and do not hydrolyze urea [46]. E. coli is a natural and essential part of the bacterial flora in the gut of humans and animals. Most E. coli strains are nonpathogenic and reside harmlessly in the colon. However, certain serotypes do play a role in intestinal and extra-intestinal diseases, such as urinary tract infections [47].

6.1.1 Pathogenic Escherichia coli Strains

*E. coli* strains isolated from intestinal diseases have been grouped into at least six different main groups, based on epidemiological evidence, phenotypic traits, clinical features of the disease and specific virulence factors. From these, enterotoxigenic (ETEC, namely O148), enterohemorrhagic (EHEC, namely O157) and enteroinvasive serotypes (EIEC, namely O124) are of outstanding importance and can be transmitted through contaminated water [47, 48].

6.1.1a Enterotoxigenic*E. coli* (ETEC) Strains

Enterotoxigenic*E. coli* (ETEC) serotypes can cause infantile gastroenteritis. The number of reports of their occurrence in developed countries is comparatively small, but it is an extremely important cause of diarrhea in the developing world, where there is no tolerable clean water and poor sanitation. Disease caused by ETEC follows ingestion of contaminated food or water and is characterized by riotous watery diarrhea lasting for several days that often leads to dehydration and malnutrition in young children. ETEC also are the most common cause of travelers diarrhea that affects individuals from industrialized countries travelling to developing regions of the World [48, 49].

6.1.1b Enterohemorrhagic*E. coli* (EHEC) Strains

These organisms produce a toxin known as verocytotoxin which is similar to the toxin produced by Shigella. Infection with this organism is associated with haemorrhagic colitis. In a small proportion of the cases, particularly in children, the infection can progress to haemolytic uraemic syndrome, a life threatening disease. *E. coli* serotype O157:H7 causes abdominal pain, bloody diarrhea, and hemolytic uremic syndrome. Although *E. coli* O157:H7 is not usually a concern in treated drinking water, outbreaks involving expenditure of drinking water contaminated with human sewage or cattle feces have been documented. An increasing number of outbreaks are associated with the expenditure of fruits and vegetables (sprouts, lettuce, coleslaw, salad) contaminated with feces from domestic or wild animals at some stage during cultivation or handling. EHEC has also been isolated from bodies of water (ponds, streams), wells and water troughs, and has been found to survive for months in muck and water-trough sediments [45,49]. Person-to-person contact is an important mode of transmission through the oral-fecal route.

6.1.1c Enteroinvasive*E. coli* (EIEC) Strains

Enteroinvasive*E. coli* (EIEC) are capable of invading and multiplying in the intestinal epithelial cells of the distal large bowel in humans. The illness is characterized by abdominal cramps, diarrhea, vomiting, fever, chills, a generalized malaise, and the appearance of blood and mucus in the stools of infected individuals. [47, 48]. An investigation in Croatia showed that *E. coli*
O124 could frequently be isolated from cases of gastroenteritis, enterocolitis, and dysentery. The dysentery was more common among the older age groups, while the two other types of disease occurred equally in all age groups. Any food contaminated with human feces from an ill individual, either directly or via contaminated water, could cause disease in others. Outbreaks have been associated with hamburger meat and unpasteurized milk.

6.2 Salmonella

Salmonella was discovered between the illness and expenditure of water from an aqueduct that flowed near the camp. The risk of suffering from the illness rose with the amount of water consumed. Chemical and bacteriological analyses of the aqueduct water indicated the presence of fecal contamination. An outbreak of gastroenteritis due to S. ohio whose origin was the expenditure of water from a drinking fountain was described for the first time by [50]. This fountain had no chlorination system. S. ohio was isolated from the water and from 2 of the 13 stool specimens analysed. A molecular epidemiology study of Salmonella serotype Enteritidis was carried out by ribotyping and randomly amplified polymorphic DNA (RAPD) typing of 38 food and 25 water strains, which were epidemiologically unrelated and collected in Spain from 1985 to 1996 [51] Their results supported the fact that organisms representing at least 40 genomic groups are currently circulating in Spain but that only the organisms of five groups preponderant and these fall into a single subcluster or lineage. Organisms of four infrequent groups were only collected from sewage or environmental waters.Typhoid fever, a severe systemic illness transmitted through food or water, is caused by the bacterium Salmonella serotype typhi. Luby et al. [52] evaluated risk factors for developing typhoid fever in a setting where the disease is endemic (Karachi, Pakistan). Eating ice cream, eating food from a roadside cabin during the summer months, taking antimicrobials in the 2 weeks preceding the onset of symptoms, and drinking water at the worksite were all independently associated with typhoid fever.

6.3 Shigella

Children under 5 years of age infected with either Shigella dysenteriae type I or Shigellaflexneri attending a diarrhea treatment center from 1993 to 1995 in Dhaka, Bangladesh. Use of antibiotics at home, use of water from tube wells or pipe-water for drinking, and lack of sanitary facilities were the behavioral and environmental factors strongly associated with S. dysenteriae type I infection. Tshimanga et al[53] investigated a July 1994 outbreak of Shigella dysenteriae type I at a textile factory in Bulawayo, Zimbabwe. Thirty seven of 58 workers who drank borehole water were ill compared to 1 of the 17 who did not. Water samples from the two boreholes yielded numerous fecal coliforms.

6.4 Vibrio

Cholera, caused by certain strains of Vibrio cholerae, is the first disease for which a waterborne route of transmission was shown. Paneth et al[54] reviewed the history of John Snow's 1854 investigation proving a waterborne route of transmission for cholera. Epidemic cholera is caused by toxigenic strains of V. cholerae, of which strains 01 and 0139 are associated most often, but not exclusively, with epidemic outbreaks. Faruque et al[55] reviewed the epidemiology, genetics, and ecology of toxigenic V.cholerae. They emphasized the close connexion among V. cholera, surface water, and the population interacting with the water. They also noted that molecular epidemiological studies have disclosed significant clonal diversity among toxigenic strains and continual Egression of new epidemic clones.

6.5 Campylobacter

Campylobacter spp. are now recognized as a major cause of gastroenteritis associated with the ingestion of contaminated food and water. Furtado et al[56] reported that Campylobacter was associated with the majority of waterborne disease outbreaks from private water supplies. Hazeleger et al.[57] examined the physiological activities of Campylobacter at several environmental temperatures. Cellular activity, and hence continuity, could be measured at temperatures as low as...
4°C. In addition, the organism was capable of chemotaxis and aerotaxis at all temperatures and thus may be able to move to more favourable environments. Both osmolality and temperature were found to be significant factors in the continuity of *Campylobacter spp.* [58]. None of the Campylobacter examined (*C. jejuni*, *C. lari*, and *E. coli*) grew in media with an osmolality of 130 mosmol and a temperature below 42°C. In media with low osmolalities, the number of viable cells declined rapidly at any of the temperatures examined.

### 6.6 Arcobacter

*Arcobacter spp.* isolated from the treatment plants showed the same serotypes as described for human isolates. Therefore, the spread of Arcobacter via the drinking water path must be suspected. *Arcobacter* enrichment medium (AM), newly developed by Oxoid, was compared with two *Campylobacter* enrichment media (Preston broth [Oxoid] and LabM broth) and with Arcobacter basal medium (ABM) as a control by [59] Atabay and Corry. Twenty strains of *Arcobacter* and *Campylobacter spp.* were tested for growth, with target inocula of less than 4 CFU/mL of medium. None of the *Campylobacter spp.* grew in the complete AM, and only one grew (very poorly) in the ABM. However, AM supported good growth of all three species of Arcobacter (*A. butzleri*, *A. skirrowii*, and *A. cryaerophilus*), which have been associated with human and animal disease.

### 6.7 Helicobacter pylori

*Helicobacter pylori* has been cited as a major etiologic agent for gastritis and has been implicated in the pathogenesis of peptic and duodenal ulcer disease and gastric carcinoma. *H. pylori* has not been isolated from environmental sources, including water [60]. On the contrary, molecular methods have been successful in detecting this pathogen. Fluorescence in situ hybridization has been successfully used to detect this pathogen in drinking water distribution systems and other water bodies. Polymerase chain reaction has also been used to detect the presence of *H. pylori* DNA in drinking water, especially associated with biofilms [61]. In drinking-water biofilms, *H. pylori* cells rapidly lose culturability, entering a viable but non-culturable state. How the organism is transmitted is still not fully understood. However, the fact that it has been recovered from saliva, dental plaques, the stomach, and fecal samples strongly indicates oral-oral or fecal-oral transmission. Water and food appear to be of lesser direct importance, but they can still play a significant role in situations with improper sanitation and hygiene [45]. The survival of *Helicobacter pylori* in artificially contaminated milk and tap water was investigated by Fan et al. [62]. *Helicobacter pylori* could survive for up to 10 days in milk at 4°C storage but only 4 days in tap water with a steady decrease of colony-forming units. However, electron microscopy clearly showed that the nonculturable coccoid form was present in tap water that had been kept at 4°C for 7 days.

### 6.8 Mycobacterium avium complex

*Mycobacterium avium complex (MAC)* organisms have been isolated from water and soil. It is now generally accepted that environmental sources, especially natural waters, are the reservoirs for most human infections caused by MAC. A typical mycobacteria are responsible for a variety of diseases, particularly in immune compromised individuals. [63] Lin et al. found that *Mycobacterium avium* was signifi cantly more resistant to disinfection with copper-silver ions than was *Legionella pneumophila*. Water, both in the city water supply and hospital environment, was found to be the major source of transmission of *Mycobacterium xenopi*, an opportunistic pathogen that causes pulmonary infections [64]. Steinert et al. (1998b) [65] compared that the growth of *Mycobacterium avium* in coculture with the free-living amoeba Acanthamoebapolyphaga with the growth of *M. avium* when it was separated from amoebae. Although viable mycobacteria were observed within amoebal vacuoles, there was no significant difference between bacterial growth in coculture and bacterial growth separately. Mac organisms have ability to survive and grow under varied conditions. Mac organisms can proliferate in water at temperatures up to 51°C and can grow in natural waters over a wide pH range [45]. These mycobacteria are highly resistant to chlorine and the other chemical disinfectants used for the treatment of drinking-water. Standard drinking-water
treatments will not eliminate Mac organisms but, if operating satisfactorily, will significantly reduce the numbers that may be present in the source water to a level that represents a negligible risk to the general population. The entryway of these mycobacteria in distribution systems is through leaks. Growth of Mac organisms in biofilms is probably important for their continuous presence in distribution systems. The symptoms encountered with Mac infections result from colonization of either the respiratory or the gastrointestinal tract, with possible diffusion to other locations in the body. Exposure to Mac organisms may occur through the expenditure of contaminated food, the inhalation of air with contaminated soil particles, or contact with or ingestion, aspiration, or aerosolization of potable water containing the organisms [45]. With respect to water supplies, infection with M. avium and M. intracellulare has been well documented. Unlike gastrointestinal pathogens, where E. coli can be used to indicate their potential presence, no suitable indicators have been identified to signal increasing concentrations of Mac organisms in water systems [45].

VII. FECAL BACTERIA IN THEIR HOSTS AND IN THE ENVIRONMENT

7.1 Bacteroides

Bacteroides are Gram-negative, non-sporeforming, anaerobic pleomorphic rods. Bacteroides are the most abundant bacteria in human feces. In animal feces, on the contrary, Bacteroides are present at low numbers. Although anaerobic, Bacteroides are among the most tolerant to oxygen of all anaerobic human gastrointestinal species. B. thetaiotaomicron is one of the most abundant species in the lower regions of the human gastrointestinal tract. Bacteroides have a high pathogenic potential and account for approximately two-thirds of all anaerobes isolated from clinical specimens. The most frequently isolated species has been B. fragilis. The survival of Bacteroides in environmental waters is usually much lower than the survival of coliforms [66].

7.2 Eubacterium

Eubacterium are anaerobic non-sporeforming Gram-positive rods. Some species have been transferred to other genera-Actinobaculum, Atopobium, Collinsella, Dorea, Eggerthella, Mogibacterium, Pseudoramibacter and Slackia. Cells are not very aerotolerant. Species isolated from the human gastrointestinal tract include: E. barkeri, E. biforme, E. contortum, E. cylindroides, E. hadrum, E. limosum, E. moniliforme, E. rectal and E. ventricosum.

7.3 Bifidobacterium

Bifidobacteria are Gram-positive, non-sporeforming, pleiomorphic rods. The optimum growth temperature is 35-39 °C. The genus Bifidobacterium contains ca. 25 species, most of which have been detected in the human gastrointestinal tract. Bifidobacteria are present in high numbers in the feces of humans and some animals. Several Bifidobacterium species are specific either for humans or for animals. Bifidobacteria have been found in sewage and polluted environmental waters, but appears to be absent from unpolluted or pristine environments such as springs and unpolluted soil. This results from the facts that upon introduction into the environment bifidobacteria decrease appreciably in numbers probably due to their stringent growth requirements. Bifidobacteria grow poorly below 30 °C and have stringent nutrient requirements. Reports on the survival of bifidobacteria in environmental waters indicate that their survival is lower than that of coliforms (Biavati, Mattarelli, 2003 and Wilson UK, 2005). The presence of bifidobacteria in the environment is therefore considered an indicator of fecal contamination. Since some species are specific for humans and animals, the identification of Bifidobacterium species present in the polluted water could, in principle, provide information on the origin of fecal pollution. Bifidobacteria are the less studied of all fecal bacteria, due to the technical difficulties in their isolation and cultivation. Other Gram-positive bacteria, such as Streptococcus and Lactobacillus, which may occur in higher numbers than bifidobacteria, can inhibit their growth. Although selective media has been designed for the isolation of bifidobacteria from environmental waters, the outcome is still unsatisfactory, with appreciable numbers of false positives and low recovery percentages (Biavati, Mattarelli, 2003 and Wilson UK, 2005).
7.4 Clostridia

The genus Clostridium is one of the largest genera of the prokaryotes containing 168 validly published species. Clostridia are Gram-positive rods, forming endospores. The genus Clostridium includes psychrophilic, mesophilic, and thermophilic species. Most of the clostridial species are motile with peritrichous flagellation. Cells are catalase-negative and do not carry out a dissimilatory sulphate reduction. Clostridia usually produce mixtures of organic acids and alcohols from carbohydrates and proteins. Many species are saccharolytic and proteolytic. Some species fix atmospheric dinitrogen. The major role of these organisms in nature is in the degradation of organic material to acids, alcohols, CO2, H2, and minerals. Frequently, a butyric acid smell is associated with the proliferation of clostridia. The ability to form spores that resist dryness, heat, and aerobic conditions makes the clostridia ubiquitous (Wilson UK, 2005, and Hippelet et al, 2003). Most species are obligate anaerobic, although tolerance to oxygen occurs. Oxygen sensitivity restricts the habitat of the clostridia to anaerobic areas or areas with low oxygen tensions. Growing and dividing clostridia will, therefore, not be found in air saturated surface layers of lakes and rivers or on the surface of organic material and soil. Clostridial spores, however, are present with high probability in these environments, and will germinate when oxygen is exhausted and when appropriate nutrients are present (Wilson UK, 2005 and Hippelet et al, 2003). C. perfringens ferment lactose, sucrose and inositol with the production of gas, produce a stormy clot fermentation with milk, reduce nitrate, hydrolyze gelatin and produce lecithinase and acid phosphatase. The species is divided into five types, A to E, on the basis of production of major lethal toxins (Rainey et al, 2009 and Smith, 2003). C. perfringens appears to be a universal component of the human and animal intestine, since has been isolated from the intestinal contents of every animal that has been studied. Humans carry C. perfringens as part of the normal endogenous flora.

7.5 Lactobacillus

Lactobacilli are non-sporeforming Gram-positive long rods. There are more than thirty species in the genus. Most are microaerophilic, although some are obligate anaerobes. Cells are catalase-negative and obtain their energy by the fermentation of sugars, producing a variety of acids, alcohol and carbon dioxide. Lactobacilli have complex nutritional requirements and in agarized media may need the supplementation with aminoacids, peptides, fatty-acid esters, salts, nucleic acid derivatives and vitamins. Lactobacilli very rarely cause infections in humans (Wilson UK, 2005).

7.6 Enterococci

Enterococci are Gram-positive, non-sporeforming, catalase-negative ovoid cells. Cells occur singly, in pairs or short chains. Optimal growth for most species is 35–37 °C. Some will grow at 42–45 °C and at 10 °C. Growth requires complex nutrients but is usually abundant on commonly used bacteriological media. The enterococci are facultative anaerobic but prefer anaerobic conditions (Wilson UK, 2005 and Švec, P.; Devriese, L.A, 2009). The genus was separated from Streptococcus in the 1980s. Enterococci form relatively distinguishable groups. Members of such groups exhibit similar phenotypic characteristics and species delimitation can be difficult. Enterococci are naturally present in many kinds of foods, especially those of animal origin such as milk and milk products, meat and fermented sausages. Enterococci are usually considered secondary contaminants of food, although they often play a positive role in ripening and aroma development of some types of cheeses [66, 67] (Wilson UK, 2005 and Švec, P.; Devriese, L.A, 2009). Although soil is not a natural habitat for enterococci, cells can be found in this habitat due to the transport by rain.

7.7 Citrobacter

Citrobacter, a member of Enterobacteriaceae, are motile straight rods. Cells are oxidase-negative, catalase-positive and positive in the Methyl-Red test. Cells use citrate, are negative in the Voges-Proskauer test and do not decarboxylate lysine [34] (Bergery’s Manual, 1994). Citrobacter species can be isolated from different clinical sites. In particular, C. freundii is intestinal inhabitants of humans that may sometimes have evolved the ability to produce an enterotoxin and thus become
an intestinal pathogen. Citrobacter is reported to occur in environments such as water, sewage, soil and food [68] (Frederiksen et al, 2003).

7.8 Klebsiella and Raoultella

*Klebsiella* and *Raoultella* are Enterobacteriaceae, oxidase-negative catalase-positive non-motile straight rods, surrounded by a capsule. Cells decarboxylate lysine, but are ornithine and arginine dihydrolase negative. Cells grow on KCN, do not produce H2S and ferment most carbohydrates [34] (Bergey’s Manual, 1994). *Klebsiellae* are ubiquitous in the environment. They have been found in a variety of environmental situations, such as soil, vegetation, or water, and they influence many biochemical and geochemical processes. They have been recovered from aquatic environments receiving industrial wastewaters, plant products, fresh vegetables, food with a high content of sugars and acids, frozen orange juice concentrate, sugarcane wastes, living trees, and plants and plant byproducts. They are commonly associated with wood, sawdust, and waters receiving industrial effluents from pulp and paper mills and textile finishing plants. Klebsiella have been isolated from the root surfaces of various plants. *K. pneumoniae, K. oxytoca*, and *R. planticola* are all capable of fixing dinitrogen [69] (Grimont et al, 2005).

7.9 Enterobacter

*Enterobacter* a member of Enterobacteriaceae, are motile straight rods. Cells are positive in the Voges-Proskauer test VP and in Simmons citrate agar. Cells do not decarboxylate lysine, but are ornithine positive. Malonate is usually utilized and gelatin is slowly liquefied. Cells do not produce H2S, deoxyribonuclease and lipase [34] (Bergey’s Manual, 1994). Before the widespread use of antibiotics, *Enterobacter* species were rarely found as pathogens, but these organisms are now increasingly encountered, causing nosocomial infections such as urinary tract infections and bacteremia. *Enterobacter* species were the second most common gram-negative organism, behind *Pseudomonas aeruginosa*. Both bacteria were reported to each represent 4.7% of bloodstream infections in intensive care units. Enterobacter species represented 3.1% of bloodstream infections in non-intensive care units. They found Enterobacter species to be the eighth most common cause of healthcare-associated infections (5% of all infections) and the fourth most common gram-negative cause of these infections [70] (Hidron et al, 2008). An Enterobacter cloacae subsp. cloaca (E. cloacae) occurs in the intestinal tracts of humans and animals, in hospital environments, the skin, in water, sewage, soil, meat. Nitrogen-fixing strains have been isolated from the roots of rice plants. *E. ammigenus* has been mostly isolated from water, but some strains were isolated from clinical specimens from the respiratory tract, wounds and feces. *E. asburiae* strains were isolated from clinical specimens, mostly urine, respiratory tract, feces, wounds, and blood [69] (Grimont et al, 2003).

VIII. CONCLUSION

1. It was concluded that safe drinking water for all is one of the major challenges of the 21st century and that microbiological control of drinking water should be the norm everywhere.

2. In this review a general characterization of the most important enteric bacteria transmitted through water is presented, focusing on the biology and ecology of the causal agents and on the diseases characteristics.

3. Currently, it is thought that the input of antibiotics in general as well as from hospitals seems to be of minor importance, at least in terms of resistance. Up to now, antibiotics have not been detected in drinking water.

4. There is insufficient information available to reach a final conclusion on the significance and impact of the presence of resistant bacteria in the environment, which would allow the assessment of the potential risks related for instance, to human health and ecosystem functions.

5. The impact of antibiotics present in the aquatic environment on the frequency of resistance transfer is questionable.
6. The present date suggests that the input of resistant bacteria into the environment from different sources seems to be the most important source of resistance in the environment. Therefore, the prudent use of antibiotics and disinfectants will significantly reduce the risk for the general public and for the environment.

7. This not only means limiting the duration of selective pressure by reducing the treatment period and the continuous use of sub-therapeutically concentrations, but also includes controlling the dissemination of antibiotics being used, as well as prudent monitoring of resistance.

8. However, a full environmental risk assessment cannot be performed on the basis of the data available; the availability of such data is a prerequisite if proper risk assessment and risk management programs for both humans and the environment are to be undertaken. Therefore, the careful use of antibiotics and the restriction of their input into the aquatic environment are the matters of necessity.

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