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IDENTIFICATION AND ANTIBIOTIC RESISTANCE OF BACTERIA ISOLATED FROM SHRIMPS

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This study aimed to identification and determines the pattern of antibiotic resistance bacteria isolated from shrimps. The susceptibility of 97 bacterial isolates to 16 different antibiotics was investigated by agar diffusion method. The most common species isolated from the samples were *Stenotrophomonas maltophilia* (19.6%), followed by *Acinetobacter lwoffi* (14.4%) and *Proteus vulgaris* (9.3%). There was a high incidence of resistance to cefazolin (100%), cefuroxime (97.9%), nitrofurantoin (97.9%) and ampicillin (96.9%), and a low incidence of resistance to cefepime (7.2%) and meropenem (22.7%) was found among selected isolates which represented the resistant bacterial population. Multiple antibiotic resistance indices ranged from 0.2 to 0.81, suggesting exposure to antibiotic contamination. These results suggest that shrimps of Iskenderun Bay have important proportion of antibiotic resistant bacteria and these bacteria can be responsible a potential risk for public health. At the same time, the finding in the aquatic environments of different combinations of resistance genes suggests their involvement in the spread of multiple antibiotic-resistant strains.

Keywords: Antibiotic resistance, MAR index, Shrimp.

Introduction

Widespread of antibiotic usage for treatment of infectious diseases prompting an extensive spread of multidrug-resistant bacteria in various environments including the aquatic environment [1]. In developing countries, antibiotics are extensively applied in aquaculture. In the aquatic environments, the presence of antibiotic resistant pathogen bacteria belonging to the Gram negative group is most important issue for public health. Hospitals discharge large quantities of antibiotic wastes in to the aquatic environment. Most of the antibiotic persist in the sediment of the aquatic environment for long time (sometimes several months) which has lead to an increase in bacteria having multiple antibiotic resistances. Resistance to several antibiotics among the bacteria, complicate treatment of infectious diseases. Fluvial waters receive human and animal wastewater discharges, which are expected to contain anti-microbial agents likely to exert a selective pressure and commensally resistant bacteria capable of transferring their resistances to autochthonous bacteria [2].

These bacteria are able to spread their resistance genes to water-indigenous bacteria. Pathogenic bacteria associated with waterborne diseases include *Vibrio cholerae*, *Vibrio parahaemolyticus*, pathogenic *Escherichia coli*, *Shigella* spp., *Salmonella* spp., *Listeria monocytogenes*, *Campylobacter* spp., *Clostridium botulinum*, *Aeromonas hydrophila*, *Yersinia enterocolitica*, *Legionella pneumophila*, *Helicobacter pylori*, and *Leptospira interrogans*. These bacteria have several ways of infecting humans, including by ingestion, inhalation or contact with a wound, and the World Health Organisation (WHO) estimates that 3.4 million people, mostly children, die from water-related diseases every year [3]. It is important to note that bacterial plasmids carry genes that code for resistance to both antibiotics and heavy metals [4]. In the past 25 years, only two new cephalosporin-beta-lactamase inhibitör combinations-ceftolozane/tazobactam in 2014 and ceftazidime/avibactam in 2015 have been approved to treat systemic bacterial infections caused by multi-drug resistant Gram-negative bacteria [5].

In the present study, it determined the prevalence of and resistance to antibiotic agents by Gram-negative bacteria isolated from shrimp collected from Iskenderun Bay, Turkey. The specific aims of this study were: (i) to identify Gram-negative bacterial strains isolated from shrimps in Iskenderun Bay; (ii) to determine antibiotic resistance of the Gram-negative bacterial strains isolated; and (iii) to determine the levels of MAR index in these strains.

Material and Methods

Penaeid shrimp samples were obtained from fisherman and packed in sterile bags. All samples were brought to the laboratory in an ice chest, and processed within 4 h of collection.

Intestinal contents of shrimps were taken aseptically, and 1 g of this was homogenised in 9 ml sterile water, the homogenised samples were then diluted using 10-fold serial dilution up to 10^7 in sterile water. The isolates were purified onto McC agar and then maintained in nutrient agar (Oxoid). All the isolates were characterised by phenotypical characteristics, namely Gram staining, oxidase and catalase reactions, motility, OF glucose and gelatin liquefaction tests according to Lemos et al. [6]. Isolates were then identified using the Becton Dickinson Crystal E/NF identification software (BBL, Md, USA). Susceptibility testing was performed by an agar diffusion test [7], using Mueller–Hinton agar (Difco)

and 16 antibiotic discs representing 9 classes of antibiotics: amikacin (AN, 30 μg), ampicillin (AM, 10 μg), nalidixic acid (NA, 30 μg), chloramphenicol (C, 30 μg), tetracycline (TE, 30 μg), nitrofurantoin (F/M, 300 μg), streptomycin (S, 10 μg), gentamicin (GM, 10 μg), imipenem (IPM, 10 μg), cefazolin (CZ, 30 μg), ceftizoxime (ZOX, 30 μg), meropenem (MEM, 10 μg), cefuroxime (CXM, 30 μg), cefepime (FEP, 30 μg), kanamycin (K, 30 μg) and trimethoprim-sulphamethoxazole (SXT, 1.25 and 23.75 μg). For all isolates, we calculated the MAR index values [8].

Results and Discussion

A total of 97 isolates was obtained representing 13 Gram-negative bacterial genera and 20 species from intestinal content of shrimps. The frequency of the isolates from shrimps is shown in Table 1. Six species were found at relatively high frequencies: *Stenotrophomonas maltophilia* (19.6%), *Acinetobacter lwoffi* (14.4%), *Proteus vulgaris* (9.3%), *Proteus penneri* (8.3%), *Burkholderia cepacia* (8.3) % and *Pseudomonas aeruginosa* (7.2%). Most isolated bacteria *Stenotrophomonas maltophilia*, a non-fermentative, Gram-negative, rod-shaped bacterium is abundant in the environment with a wide geographical distribution. This bacterial species has been isolated from aqueous sources, both in and out of clinical settings [9].

Stenotrophomonas maltophilia is an important multidrug-resistant nosocomial pathogen associated with high mortality especially among immunocompromised patients. Infections caused by S. maltophilia have a high attributable mortality rate (37.5 %) [10].

Table 1. Distrubition of Gram (-) bacterial isolated from shrimps

Species	No	%
Acinetobacter bzumannii	3	3.1
Acinetobacter lwoffi	14	14.4
Burkholderia cepacia	8	8.3

Chryseobacterium indologenes	2	2.1
Chryseobacterium meningosepticum	1	1.0
Edwardsiella tarda	2	2.1
Empedobacter brevis	1	1.0
Enterobacter cloacae	2	2.1
Enterobacter sakazakii	1	1.0
Flavimonas oryzihabitans	5	5.2
Pantoea agglomerans	1	1.0
Proteus penneri	8	8.3
Proteus vulgaris	9	9.3
Pseudomonas aeruginosa	7	7.2
Pseudomonas fluorescens	4	4.2
Pseudomonas putida	3	3.1
Pseudomonas stutzeri	4	4.1
Shigella species	1	1.0
Sphingomonas paucimobilis	2	2.1
Stenotrophomonas maltophilia	19	19.6
Total	97	100

Among the isolates, a high percentage of bacteria were resistant to cefazolin (100%), nitrofurantoin (97.9%), cefuroxime (97.9%), and ampicillin (96.9%). As shown Figure 1., a high percentage of bacteria were resistance to carbapenems (imipenem (27.8%) and meropenem (22.7%)) whereas, a low percentage of bacteria were resistance to fourth generation of cephalosporins (cefepime (7.2%)).

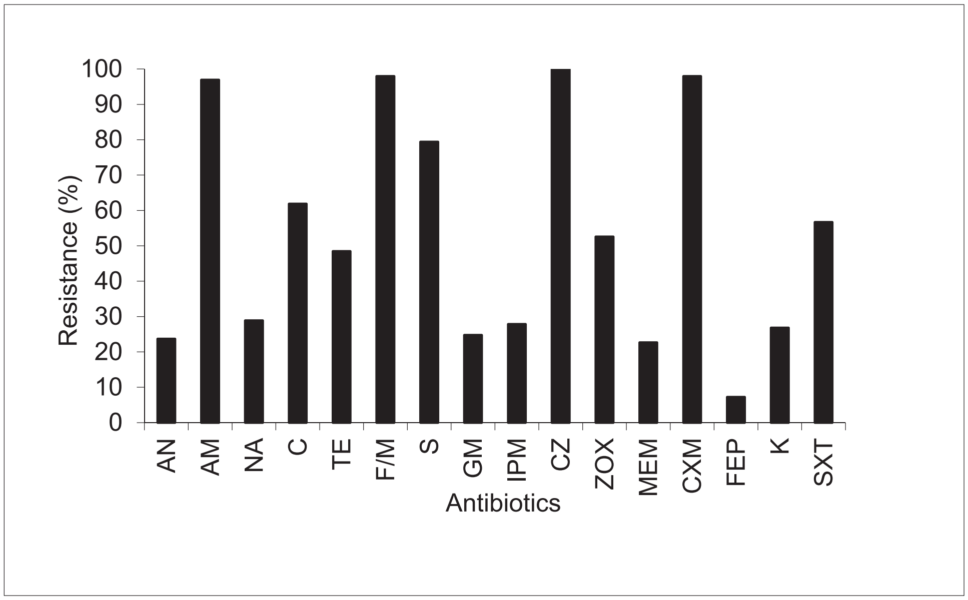

Figure 1. Antibiotic resistance of bacteria isolated from shrimp

The use of antibiotics to control infectious diseases in humans and livestock is increasing steadily worldwide, and the uncontrolled release of antibiotics coupled with industrial pollution may cause an increase in antibiotic- and heavy-metal-resistant bacteria. In particular, hospitals discharge large quantities of untreated antibiotic waste into the environment, which has contributed to the emergence of bacteria with multiple antibiotic resistances and increased virulence [11]. The increase in antibiotics resistance for some pathogens is a significant global problem, complicating the battle against infectious diseases. Some of these microorganisms are found in fishery products, which have a very crucial role in food chain, and this may pose important public health problems [12].

The MAR index ranged from 0.25 to 0.81 for the isolates (Figure 2). Matyar at al. [13]. reported that the MAR index ranged from 0.25 to 0.81 for Gram-negative bacteria in their study performed in Adana, Turkey

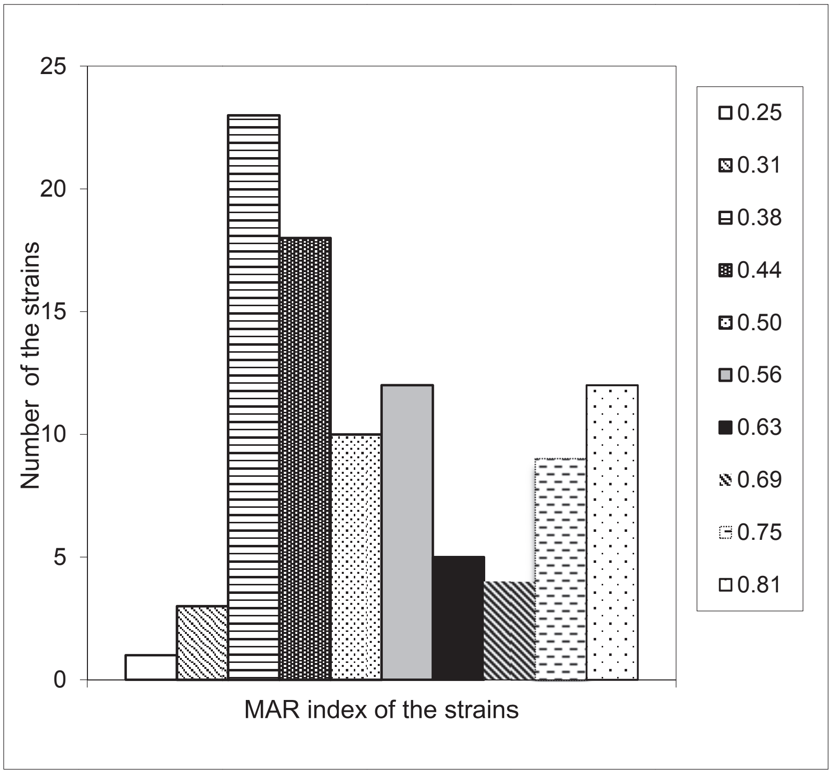

Figure 2. MAR index of bacteria isolated from shrimp

Conclusion

Increasing antibiotic resistance among the pathogen bacteria, leads to significant difficulties in the treatment of infectious diseases. Environmental pollution can be accompained by resistance of aquatic organisms to various antimicrobials. Seafoods are usually consumed after being cooked in Turkey, and therefore, shrimp may be a low risk food, even if contaminated with enteric bacteria species. However in recent years, the trend of consuming ready to eat raw seafood in public places is getting popular and thus, there is always the possibility of cross-contamination at processing food preparation and service steps. The presence of high numbers of antibiotic-resistant bacteria in shrimps may have ecological and public health implications.

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