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Study of biofilm formation in *Salmonella* species isolated from food

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ABSTRACT

Objectives: Biofilms are defined as communities of organisms attached to a surface and producing an extracellular matrix, in which the bacteria are imbedded. Infections with *Salmonella* species represent a major health problem and a significant burden on food industry. Biofilm formation is one of the causes of pathogenicity of *Salmonella* species, especially in the food industry, which allows bacteria to bind to different levels. Many outbreaks have been associated with biofilms, because they quickly resist anti-microbial and cleansing agents. The aim of this research was to study the capability of biofilm formation by *Salmonella* species isolated from food.

Methods: A total of 8 *Salmonella* species were isolated from 400 samples of red meat, chicken, eggs, and vegetables. Identification was carried out by conventional biochemical tests and serotyping. The capability of biofilm production was measured by titration in Crystal Violet microplate.

Results: In the phenotypic study of *Salmonella* isolates with turbidity method at 550 nm without acetic acid, only 2 (25%) of isolates were able to produce biofilm. both of isolates belonged to the group D of *Salmonella*.

Conclusions: The capability of the isolates to form biofilm reveals the potential ability to resist antimicrobial chemotherapy, therefore higher levels of hygiene in production, packaging, and supply are necessary.

Keywords: *Salmonella*, biofilms, foodborne disease

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The incidence of non-typhoidal salmonellosis in the United States is reported to be 1.4 million per year, with over 95% of these cases being foodborne diseases and 30% of these food infections results in death. Various studies have shown the high capability of *Salmonella* species to bind and form biofilm on different surfaces [1, 2]. A biofilm is any group of microorganisms in which cells stick to each other and

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often also to a surface. These adherent cells become embedded within a slimy extracellular matrix that is composed of extracellular polymeric substances [3]. The formation of biofilms reduces the susceptibility to antimicrobial treatment which will ultimately lead to high treatment costs for patients [4]. Food contamination in the production line through unsanitary surfaces is one of the most common problems in food processing plants. Improperly cleaned and residue levels are a good environment for binding and growth, of pathogenic bacteria and, consequently, biofilms formation. The passage of the processed product from contaminated surfaces causes its microbial contamination [5, 6]. The growth of bacteria in the biofilm on the surfaces makes it easier them to transport and difficult to eliminate them. Because biofilm cells exhibit greater resistance to biosolids and disinfectants compared with free cells [7, 8]. The growth of biofilms on food processing equipment causes microbial contamination in the process product, thus reducing the shelf life of the product and increasing the prevalence of food-borne diseases, in particular, those related to *Listeria monocytogenes* and *Salmonella* species. These biofilm contain pathogenic microorganisms [9, 10]. Since there was little information about the formation of biofilm from *Salmonella* species isolated from food in Iran the purpose of this study was to investigate thecapability of biofilm formation by *Salmonella* species isolated from food.

METHODS

A total of 8 *Salmonella* species were isolated from 400 samples of red meat, chicken, eggs, and vegetables. Identification was carried out by conventional biochemical tests and serotyping. Antibiotic sensitivity tests were carried out on the identified *Salmonella* species by using the Kirby-Bauer (Figure 1). Twelve antibiotic discs, namely amoxicillin, nalidixic acid, chloramphenicol, imipenem, tetracycline, ciprofloxacin, ceftriaxone, meropenem, streptomycin, cefepime, cefuroxim and cotrimoxazole. Results were analyzed according to Clinical and Laboratory Standards Institute (CLSI) [11].

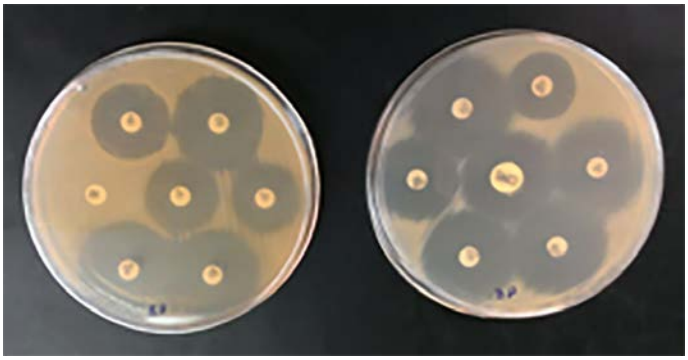

Figure 1. Antibiotic sensivity test.

Biofilm Production

The capability of biofilm production was measured by titration in Crystal Violet microplate according to the instructions used by Peeters *et al*. [12]. Samples was cultured in tryptic soy broth (TSB) and incubated at 37° C for 24 hours. After dilution in fresh TSB, 150 ml of cell suspension was poured into a 96 well flat-bottom polystyrene microplate and incubated at 37° C for 24 hours. The plate was washed three times with 200 µl of posphate-buffered saline (PBS) and air-dried. For fixation of biofilms, 100 µl of 99% methanol was used, after 15 minutes, alcohol was removed and plates were dried in air. 100 µl of 2% crystal violet was added to all of the wells and after 20 minutes the plates were washed with water to remove the color residues. The bonded colors were then released by adding 150 µL of 33% acetic acid. The light absorption (OD) of each well was measured at 570 nm using the ELISA reader. All measurements were repeated 4 times .This was repeated in three separate experiments. E. coli Top 10 and E.coli EAEC 042 strains were used as a negative and positive control respectively.

RESULTS

Of the eight isolated *Salmonella*, two isolates had the capability to produce biofilms, both of which belong to group D (Figure 2). The *Salmonella* isolates showed, the highest resistance 6 (75%) to nalidixic acid, 3 (37.5%) were intermediate to ciprofloxacin and cefuroxime amoxicillin. All isolates 8 (100%) were sensitive to chloramphenicol, imipenem, meropenem, ceftriaxone, cefepime, streptomycin, and cefotaxime. Serogroup D *Salmonella* has the highest resistance to nalidixic acid (75%). Serogroup A was susceptible to

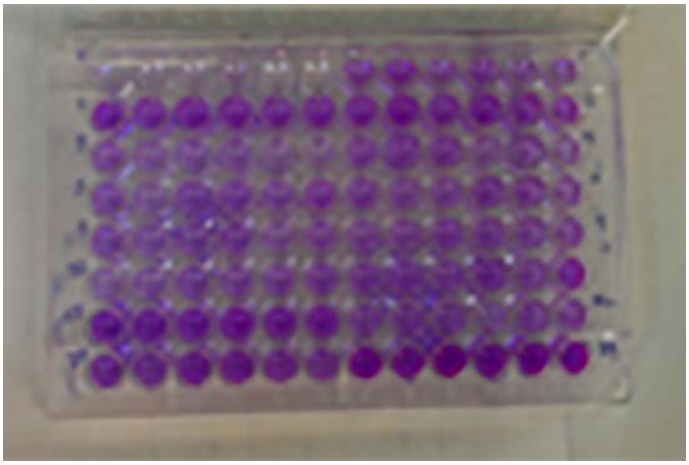

Figure 2. Biofilm production.

cefuroxime and nalidixic acid and intermediate to the rest of antibiotic. *Salmonella* serogroup B was resistant to nalidixic acid, tetracycline, cotrimoxazole, and amoxicillin and sensitive to the rest of the antibiotic. *Salmonella* serogroup C was resistant to nalidixic acid and tetracycline, intermediate to ciprofloxacin and sensitive to the rest of antibiotics. All non-typeable *Salmonella* showed 100% sensitivity to the entire tested antibiotic (Table 1).

DISCUSSION

Salmonella is an important foodborne pathogen and its prevalence in fresh food poses a threat to human. The increase in demand and consumption of raw vegetables has resulted in a rise in food-borne related illnesses and outbreaks. The biofilm formation is a mechanism of *Salmonella* to adapt to different environments. They have been of considerable interest in food hygiene since biofilms may contain spoilage and pathogenic bacteria which increases post-processing contamination and risk to public health. In addition, biofilm cells are more resistant to cleaning and disinfection processes in the food industry. A number of studies have shown that *Salmonella* spp. are capable of adhering and forming biofilms ondiverse surfaces including metal, glass and rubber surfaces [13-15]. The assessment of biofilm formation by *Salmonella* on microtitre plateshowed that all *Salmonella* isolates were able to form biofilms. Other research worker showed that the *Salmonella* were able to form biofilm on microtiter [16].

Pervious study also showed that *Salmonella* biofilms grown and established on stainless steel

Table 1. Antibiotic susceptibility profile of serogroup A, B, C & D and non-typeable *Salmonella*.

Antibiotics	Intermediate %	Sensitive %	Resistant%	Sensitive %	Intermediate	Sensitive %	Resistant%	Intermediate %	Sensitive %	Sensitive %
	serogroup A		serogroup B		serogroup C		serogroup D			non-typeable
Amoxicillin	100	0	100	0	0	0	100	100	0	100
Nalidixic acid	0	100	100	0	0	100	0	0	25	100
Chloramphenicol	100	0	0	100	0	0	100	0	100	100
Imipenem	100	0	0	100	0	0	100	0	100	100
Tetracycline	0	0	100	0	0	100	0	0	0	100
Ciprofloxacin	100	0	0	100	100	0	0	100	0	100
Ceftriaxone	100	0	0	100	0	0	100	0	100	100
Meropenem	100	0	0	100	0	0	100	0	100	100
Streptomycin	100	0	0	100	0	0	100	0	100	100
Cefepime	100	0	0	100	0	0	100	0	100	100
Cefuroxime	0	100	0	100	0	0	100	100	0	100
Cotrimoxazole	0	0	100	0	0	0	100	0	0	100

surfaces as well as meat thawing-loss broth (MTLB). This finding is a matter for concern, particularly for the poultry and meat processing industries using modern meat processing equipment. In these situations with mechanical and process automation, the surfaces are in repeated contact with raw meat, thusincreasing the opportunities for *Salmonella* transfer and attachment leading to biofilm formation [17].

The *Salmonella* isolates showed, the highest resistance 6 (75%) to nalidixic acid, 3 (37.5%) were intermediate to ciprofloxacin and cefuroxime amoxicillin. All isolates 8 (100%) were sensitive to chloramphenicol, imipenem, meropenem, ceftriaxone, cefepime, streptomycin, and cefotaxime. Serogroup D *Salmonella* has the highest resistance to nalidixic acid (75%). Serogroup A was susceptible to cefuroxime and nalidixic acid and intermediate to the rest of antibiotic. Several studies have documented high resistance of *salmonella* to the tetracyclines [18, 19], which is in agreement with the result obtained in this study. *Salmonella* serogroup B was resistant to nalidixic acid, tetracycline, cotrimoxazole, and amoxicillin and sensitive to the rest of the antibiotic. *Salmonella* serogroup C was resistant to nalidixic acid and tetracycline, intermediate to ciprofloxacin and sensitive to the rest of antibiotics. All non-typeable *Salmonella* showed 100% sensitivity to the entire tested antibiotic. A study carried in Canada showed the highest incidence of food-borne outbreaks, with the highest intake of vegetables and fresh fruits, with *Salmonella* with 50% had the highest incidence of this disease, while *Salmonella* isolates from food were 2% and vegetarians showed lower rates than chicken and meat [20]. A study a total of 48 strains of *Salmonella* enteritidis isolated from various sources in South America were investigated in terms of virulence factors including invasion, biofilm production, movement, presence of viral plasmid [21]. In this study, most strains were highly invasive and only three strains were low invasive. All the strains with low invasive did not produce biofilms, while 53% of high invasive produced biofilm [21]. In food industries, the binding of pathogenic bacteria and food corrosive to food contact levels in their production and packaging processes, and finally, the formation of microbial biofilms could be a potential source of contamination of food products and diseases and transmission of diseases. Biofilms on the surfaces of bacteria make it easier to transport and eliminate them. Because biofilm cells exhibit greater resistance to biosolids and disinfectants compared with free cells [7].

CONCLUSION

To consider the ability of producing biofilm by isolated *salmonella* from food samples and rising of *salmonella* gastroenteritis's especially group D, needing for more care and observance a higher level of health to preparation, producing, packing and supply of food seems.

Conflict of interest

The authors disclosed no conflict of interest during the preparation or publication of this manuscript.

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