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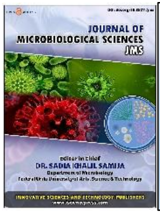
Research Article



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Isolation, Identification and Evaluation of Antibiotic Sensitivity and Resistance of *Shigella Dysenteriae* Isolated from Food, Water and Soil

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Abstract

Background: *Shigella* is a genus of gram negative, facultative anerobic rod shaped bacteria divided into four species that are *Shigella dysenteriae* (serogroup A), *Shigella flexneri* (serogroup B), *Shigella boydii* (serogroup C) and *Shigella sonnei* (serogroup D). Shigella species are the etiological agent of shigellosis and most serious illnesses are caused by *Shigella dysenteriae.*

Aim: The aim of this study was to determine antibiotic sensitivity and resistance of *Shigella dysenteriae* isolates to ensure that severe diarrhea infection remain treatable.

Method: A total of 75 samples from different environmental sources were collected, processed and tested for the identification of *Shigella dysenteriae* after observing the cultural, morphological and biochemical characteristics. Antibiotic sensitivity and resistance pattern of these isolates were also studied by disk diffusion method.

Results: It was observed that out of 75 samples, 42 samples were positive for *Shigella dysenteriae*. All *Shigella* isolates were highly resistant to Penicillin (100%), Vancomycin (100%), Aztreonam (100%), Ertapenem (100%) followed by Ceftriaxone (78%) and aAmikacin (74%) whereas highly sensitive to Imipenem (72%) followed by Ciproflaxin (67%) and Meropenem (47%).

Conclusion: Resistance of *Shigella dysenteriae* to antibiotics that are commonly used for treatment of shigellosis is increasing at alarming rate. Multipronged strategies should be adopted to ensure that severe diarrhea infection remain treatable.

Key words: Shigella; Colony morphology; Biochemical tests; Antibiotic resistance.

Introduction

Shigella is a member of the *Enterobacteriaceae* family, a common inhabitant of intestine. It is classified as Gram negative, non-motile, non-spore forming and facultative anaerobic rods measuring 0.5-0.7µm in size that cause mild to severe infections (Ud-Din and Wahid, 2014). *Shigella* is the most common cause of non-bloody and bloody diarrhea (Dooki *et al*., 2014). The pathogenicity of *Shigella* is considered to be high as only 10-100 organisms are far enough to cause infection. High resistance is shown by bacteria to the stomach acid due to which they can easily pass through this barrier (Patil and Lava, 2012). The pathogen starts multiplying in the cells and spread to neighboring cells after attachment to mucosal epithelial cells of the intestine. This causes the destruction of targeted cells reflecting the characteristic pathology of dysentery (Todar, 2009). Almost 80-165 million cases are reported with 600,000 deaths due to shigellosis around the world every year. Studies also reveal that the cases of shigellosis account for 163 million developing and 1.5 million in developed countries (Heiman and Bowen, 2013). Annually 91 million cases of shigellosis with 414,000 deaths occur in Asia alone whereas, the estimated number of cases of *shigella* infections in Africa is 8 million (WHO, 2005). *Shigella* species are responsible for causing shigellosis or bacillary dysentery. The current classification divides this genus into four species namely *Shigella dysenteriae* (serogroup A), *Shigella flexneri* (serogroup B), *Shigella boydii* (serogroup C) and *Shigella sonnei* (serogroup D) (Livio *et al*., 2014). These bacteria despite of being water and food-borne pathogen can efficiently spread from person to person in low doses by contaminated fomites (Jun *et al*., 2016). Among four species of genus *Shigella*, *S. dysenteriae* has a strongest potential for causing infection and has an inpatient mortality rate of up to 20%. It causes colitis and hemolytic uremia because of production of Shiga toxins (Kothary and Babu, 2001). *Shigella* species are responsible for causing shigellosis or bacillary dysentery. Symptoms of shigellosis may range from watery diarrhea to inflammatory dysentery (bloody and mucoid stools). Abdominal cramping, malaise, fever, nausea, convulsions and vomiting may also occur. Hemolytic uremia, joint pains, dehydration, septicemia and hypoglycemia are some of the complications associated with shigellosis (Christopher *et al*., 2010). The isolation of *Shigella* from samples such as faces or rectal swabs is helpful in diagnosing *shigella* infections (Marteyn and Sansonetti, 2012). A report issued by the public health authorities in the United States, states that shigellosis has been ranked third among bacterial infections of intestine (Nüesch-Inderbinen *et al*., 2016). Whereas, in China, the major cause of disease-related death in children is shigellosis and it is also the third most common cause of morbidity as well (Scallan *et al.,* 2011). Unsafe drinking water, unhygienic conditions and improper management of wastes contribute to shigellosis in developing nations. Shigellosis in developed countries may occur as a result of intake of contaminated food material or travelling to developing nations. It is clear that shigellosis is a serious public health problem worldwide (Colin *et al*., 2009). Sanitation, water supply and household environment including fly aggregation are some of the environmental risk factors contributing to Shigella infection (Izumiya *et al*., 2009). In case of shigellosis, antimicrobial therapy is likely to be of greatest benefit as it decreases the release of pathogens throughout course of infection as well as reduction in the duration of fever (Chompook, 2011). Antimicrobial susceptibility patterns of *Shigella* sp. vary among its serogroup or between geographical areas periodically (Phavichitr and Smith, 2003). The purpose of this study is to evaluate antibiotic sensitivity and resistance of *Shigella dysenteriae* along with its isolation and identification.

Materials and Methods

Sample Collection

A total of 75 samples from different environmental sources were collected from area of Ghazi, Chakai, Buthri dam, Khanpur dam and Bandiserah Haripur, Pakistan. All the samples were brought in falcon and forensic bags (stored at 4ºC) to the laboratory for isolation, identification and evaluation of antibiotic susceptibility of *Shigella dysenteriae* according to standard methods.

Isolation of *Shigella dysentriae*

In case of water sample, 9ml of peptone water was taken in test tube along with 1ml of water sample. While in case of soil sample master dilution was prepared then 1ml from that dilution was transferred to 9ml of peptone water. Test tubes were incubated at 37ºC for 24 hours. After 24 hours samples were streaked on *Salmonella Shigella* agar and incubated for 24 hours.

Identification of *Shigella dysentriae*

Colony morphology, gram staining and biochemical tests (catalase and oxidase test) were performed for identification. Antibiotic sensitivity tests were performed. The susceptibility of *Shigella dysenteriae* isolates to penicillin, vancomycin, amikacin, ceftriaxone, imipenem, ertapenem, ciprofloxacin, aztreonam and meropenem. Was performed by using Mueller Muller Hinton Agar. The pure culture was transferred in 1ml phosphate buffer saline (PBS) solution and vortexed for 30 seconds. The cotton swabs were then dipped in suspension and spread on Muller Hinton Agar plate followed by placing antibiotic disc at different positions. After 24 hours the zones of inhibition were measured and compared with that of the reference ranges.

Results

In the present study a total of 75 samples were examined out of which 42 samples were found to be positive for *Shigella dysenteriae*. Identification was done on different morphological, microscopic and biochemical basis. Isolates having round or irregular colonies of pink black or white colour. The pink coloured rods were observed after gram staining (Figure 1). Whereas, biochemical tests showed negative results for oxidase and positive for catalase test indicating isolates as *Shigella dysenteriae* (Table 1 and Figure 2). Antibiotic susceptibility pattern of nine different antibiotics by Disk Diffusion method showed that all *shigella dysenteriae* isolates have highly resistance to penicillin (100%), vancomycin (100%), aztreonam (100%), ertapenem (100%) followed by ceftriaxone (78%) and amikacin (74%) whereas highly sensitive to imipenem (72%) followed by ciproflaxin (67%) and meropenem (47%) (Figure 3 and 4).

Table 1: Morphological, Microscopic and Biochemical characteristics of *Shigella dysenteriae*

Tests performed	Results	Identification
Morphological Characteristics		
Colony colour	Pink / Black / White	*Shigella dysenteriae*
Colony shape	Round / Irregular	*Shigella dysenteriae*
Microscopic Observation		
Gram staining	Pink, rods	*Shigella dysenteriae*
Biochemical Characteristics		
Oxidase	#-- (no change in colour)	*Shigella dysenteriae*
Catalase	+ (bubble formation)	*Shigella dysenteriae*

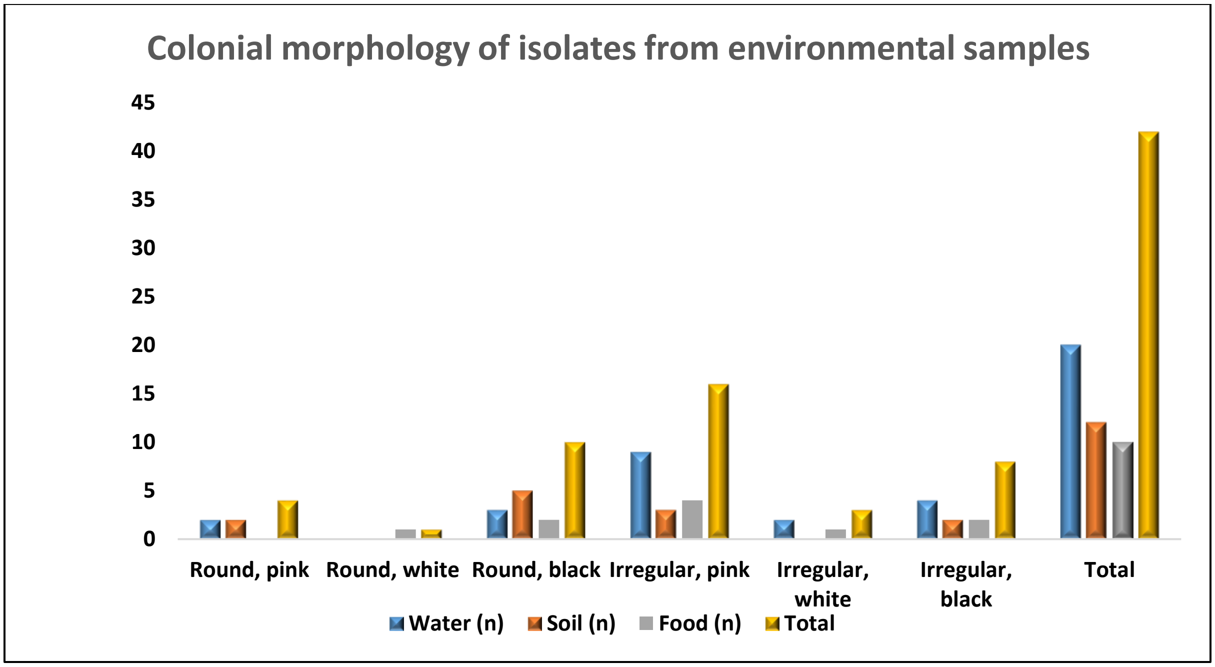


Figure 1: No. of strains identified as positive for *Shigella dysenteriae* from different environmental samples

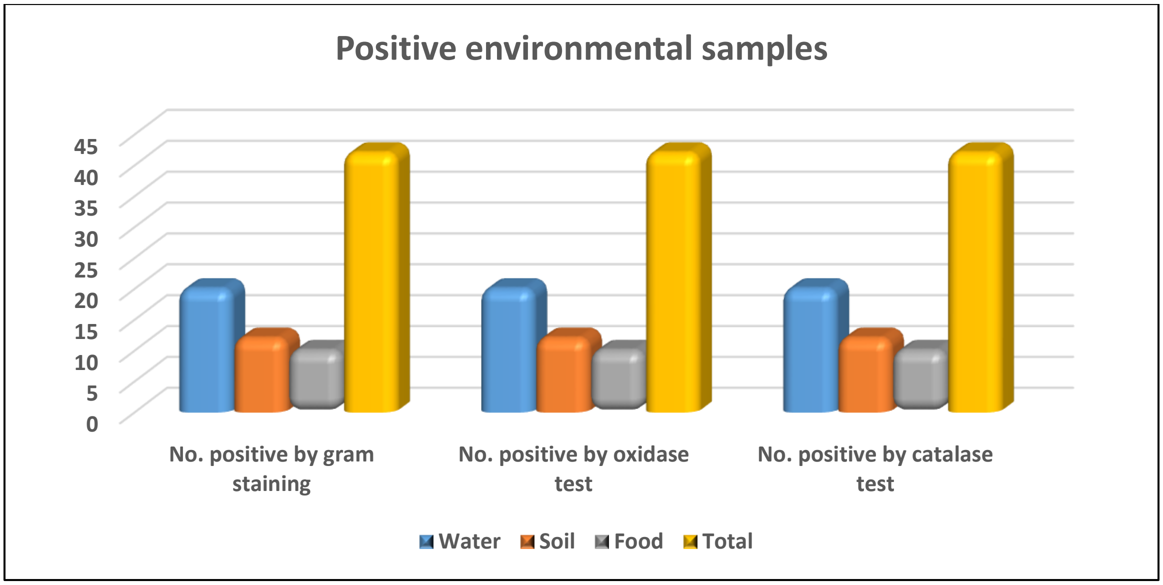
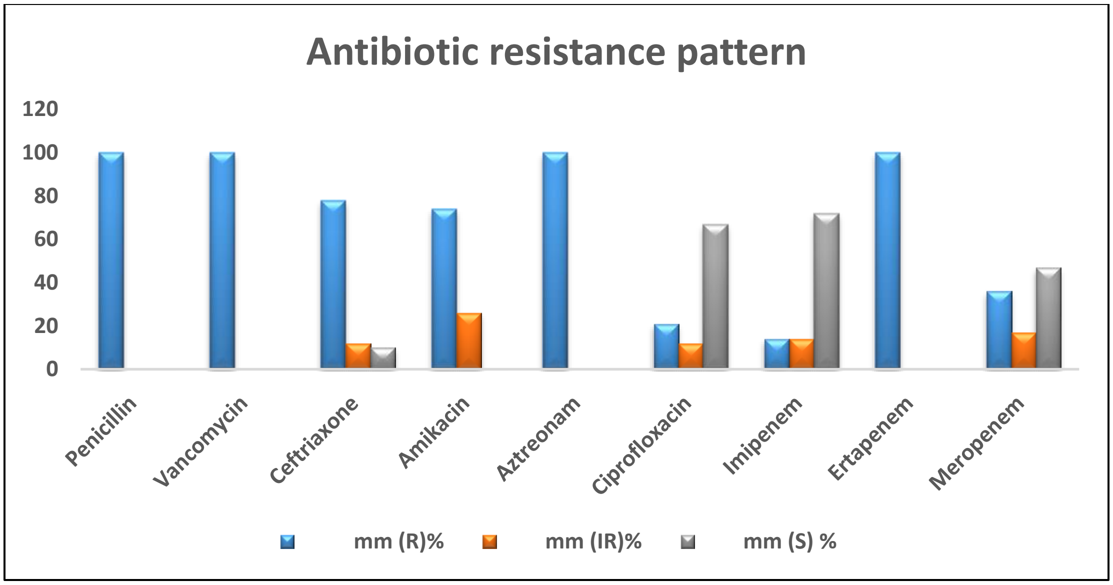


Figure 2: No. of strains identified as positive for *Shigella dysenteriae* on the basis of gram staining, oxidase test and catalase test.



Key: R= Resistant IR= Intermediate Resistant S= Sensitive

Figure 3: Antibiotic sensitivity pattern of different antibiotics used against isolates of *Shigella dysenteriae*

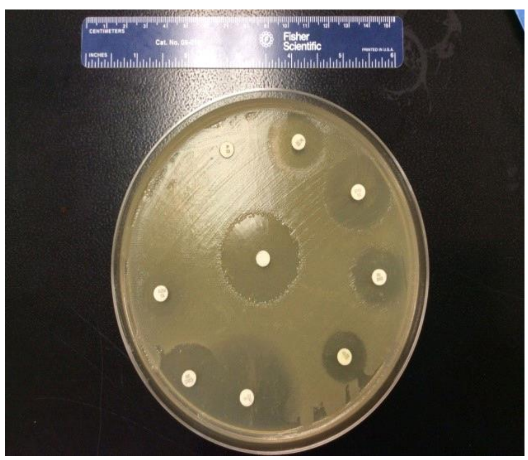


Figure 4: Antibiotics sensitivity pattern by disc diffusion method

Discussion

Across the world shigellosis accounts for a common cause of diarrhea among children. This disease exist as endemic among various developing countries. This study was carried out to find out the prevalence of *Shigella* strains in various environmental samples and their resistance and sensitivity pattern to most commonly used antibiotics.

In this study a total of 75 samples were collected from environmental sources out of which 42 samples were found to be positive for *Shigella dysenteriae*. Apart from the identification, this study also assessed the sensitivity and resistance of *Shigella* isolates to nine different antibiotics. Since the *Shigella* sp were not isolated from the laboratory samples that's why the term prescription of antibiotics for treatment cannot be used. It was found that isolates were more sensitive to imipenem (72%) that correlates with finding of Bhattacharya *et al., (*2018) who reported sensitivity of *Shigella* isolates for imipenem. The *Shigella* isolates were also found to be sensitive to ciprofloxacin (67%). The results of this study are more or less similar to the findings of Sheikh *et al.*, (2019) who have reported the sensitivity of *Shigella* isolates to ciprofloxacin up to 76.6%. Apart from the observation of sensitivity results, current study also evaluates the resistance of *Shigella* isolates for ciprofloxacin (21%). The resistance pattern is consistent with the study performed by Opintan and Newman in 2007 who observed that 25.2% of *Shigella* isolates were resistant to ciproflaxin. Similar findings by Bhattacharya *et al.,* (2005) and Samie *et al.,* (2009) revealed that the resistance to ciprofloxacin by the *Shigella* isolates were found to be 28.3 and 16% respectively. Our results also indicate that *Shigella* isolates were resistant (74%) to amikacin which is high as compared to the percentage (63%) reported by Pourakbaria *et al.,* (2010). On the other hand the values regarding ceftriaxone showed 78% resistance which were entirely different from the results presented by Muray *et al.,* in 2007. The researchers indicate a high susceptibility rate to ceftriaxone. Similarly 100% sensitivity to ceftriaxone by *Shigella* sp has been reported by Neupane *et al.,* in 2007.

Conclusion

Resistance of *Shigella dysenteriae* to antibiotics that are commonly used for the treatment of shigellosis is increasing at alarming rate and it has become harder to treat shigellosis with them. Several reasons could be accounted for this situation such as self medication, misuse of antibiotics, unnecessary and over prescribing of antibiotics.

Recommendations

Combat drug resistance: no action today means no cure tomorrow. Multipronged strategies such as minimizing unnecessary and overprescribing of antibiotics, smart use of prescribed antibiotics and continuous monitoring of antibiotic resistance should be adopted to ensure that severe diarrhea infection remain treatable. Maintaining good personal hygiene may also prevent shigellosis.

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