Profiling and Curing from *Shigella* Spp Isolated from Plasmid Diarrheal Patients

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**Abstract:** Problem statement: Shigellosis is a common infectious disease especially in underdeveloped countries. The bacteria are primarily transmitted through the faecal-oral route. The inflammatory process of acute *Shigella* infection affects the colon and is characterized clinically by fever, cramping abdominal pain with frequent loose stools that might contain mucus, pus and blood.  

**Approach:** This study determined the susceptibility of *Shigella* isolates to antibiotics. Afterward, plasmid isolation from pathogenic *Shigella* will be carried out to achieve information concerning to the presence of plasmid DNA in *Shigella* isolates. Plasmid curing will be done to seek correlation between resistance to antibiotics and plasmid occurrence in *Shigella*.  

**Results:** We found that the incidence of diarrhea in male is almost similar to female. The distribution of *Shigella* spp., in male more than in female represented 54.54 and 45.46% respectively.  

**Conclusion:** Approximately 89.5% of the diarrhea cases had no bacterial pathogen, suggested of probability of viral infection.

**Keywords:** *Shigella* spp., plasmid, antibiotic resistance, antibiotics, diarrheal patients

**INTRODUCTION**

Shigellosis is a common infectious disease especially in underdeveloped countries. WHO bulletin concluded that, 99% of the estimated 165 million cases of *Shigella* diarrhoea annually occurs in developing countries. Majority (69%) of episodes are seen in children under five years of age 3. This is attributable to personal hygiene and sanitary conditions which promote spread of organisms like *Shigella* and other enteric pathogens (Kotloff et al., 1999; Hawari, 2008).

Four serogroups (or species) of *shigella* have been described including group A (*Shigella dysenteriae*), group B (*Shigella flexneri*), group C (*Shigella boydii*) and group D (*Shigella sonnei*). These groups are further classified into serotypes and sub-serotypes. This serotyping scheme uses the polysaccharide O antigen found in the outer part of the cell wall. *Shigella* organisms are highly virulent. A very small inoculum-as little as ten microorganisms-can cause disease in humans (Ozuah, 1998; Raja et al., 2009). Antibiotic resistance among enteric pathogens is of great importance in the developing world, where the rate of diarrhoeal disease is high. Continued mismanaged selective pressure has contributed towards the emergence of multiple drug resistant bacteria and that has been regarded as an inevitable genetic response to antimicrobial therapy (Smith et al., 2003). Frequency of antibiotic resistance among *Shigella* species is growing up and has been reported in various studies globally.

The ability of a genetic marker for transferring from one bacterium to another through conjugation or transformation provides a good presumptive evidence for the involvement of plasmid, particularly if the transfer frequency is high. Moreover, loss of certain genetic markers as a result of treatment of bacterial cell to plasmid curing agents also suggests for the plasmidial nature of the marker (Mesas et al., 2004; Altalhi, 2007). The inhibition of conjugational transfer of antibiotic resistance plasmid can be exploited to reduce the spread of antibiotic resistance plasmid in the ecosystem. Inhibition of plasmid replication at various stages, as shown in the “rolling circle” model (replication, partition, conjugal transfer) may also be the theoretical basis for the elimination of bacterial virulence in the case of plasmid mediated pathogenicity and antibiotic resistance (Alhaj et al., 2008).

**MATERIALS AND METHODS**

**Sample collection:** Stool samples were collected from patients with diarrhoea admitted to the Baghdad teaching laboratory between September 2008 and December 2008. All submitted stool samples received in transport media (Phosphate buffered saline) and were inoculated on MacConkey, Xylose-Lysine Deoxycholate (XLD) agar and for enrichment in Selenite-F broth and then incubated at 37°C for 24 h in aerobic environment. After overnight incubation, Selenite-F broth was subcultured on Salmonella-*Shigella* agar.
Bacterial identification: Colonies morphologically suggestive of Shigella species were identified by conventional biochemical reactions (urea, citrate, triple sugar iron, indole, motility) and for further checked by API 20 E (Bio Murex, France).

Serotyping: Shigella isolates were grouped serologically by a slide agglutination test with antisera (Becton Dickinson and Company, Franklin Lakes, NJ, USA).

Antimicrobial susceptibility testing: Was performed with the standard disk diffusion method according to the National Committee for Clinical Laboratory Standards (2002) (NCCLS). A total of 6 antimicrobials, ampicillin, chloramphenicol, trimethoprim-sulfamethoxazole, ceftriaxone, ciprofloxacin and tetracycline, were tested.

Plasmid curing: The procedure of Zurkowski and Lorkiewicz (1978) was used for heat curing. Overnight YEM broth cultures were inoculated into PA broth. The cultures were incubated in 35, 37, 40 and 45°C water baths and given daily 5 sec. blending in a Vortex mixer for aeration. The cultures were transferred to fresh broth at weekly intervals. After heat treatment, the cultures were plated on YEM agar and the plasmid profiles of single colony isolates were observed.

Curing of plasmids by ethidium bromide: Techniques were similar as described by Ansari and Khatoon (1997). The plasmids were studied for curability in isolated colony. N.B. broth (5 mL) tubes containing graded concentration of Ethidium Bromide (EBr) were incubated with log phase cultures of Shigella isolates host bearing R plasmids to give a 20 fold dilution. A control tube lacking EBr was always included. All the tubes were incubated overnight at 37°C. The contents of the control tube and of the EBr-containing tubes were plated on MA to obtain isolated colonies. After overnight incubation at 37°C, these were replicated on antibiotics containing plates to check for the loss (or its absence) of antibiotic resistance determines.

Curing of plasmids by sub culturing: Kamiunten (1990) method used with some modified as follow. Shigella isolates had optimum growth temperature of 37°C a. Bacterial strains were grown in 5 mL of YP medium at 37°C for 24 h and then 0.1 mL of the cell suspension was transferred to 5 mL of the fresh same medium and shake-cultured at 37°C for 24 h. After twelve times of subculturing under the same conditions, the cultures were diluted with sterile distilled water and plated on YP agar medium. After 2 or 3 days of incubation at 37°C. These clones resulted from streaking on the medium were subcultured for examination of their plasmid content.

Plasmid DNA isolation: The alkaline lyses method (Kado and Liu, 1981) was used for plasmid DNA isolation.

Agarose gel electrophoresis: About 0.8% agarose in TBE buffer was used as described (Maniatis, 1982).

RESULTS

In this study four hundred patient with acute diarrhea were involved. They were 232 males (58%) and 168 females (42%). The age ranged between months to 70 years as show in (Table 1).

Like many other developing countries, diarrheal diseases are among the main health problems in Iraq. During the study period, 400 stool samples were received were found to be positive for Shigella spp., 11 isolates (2.75% from total samples), 42 samples gave positive results, but the results were canceled and focusing on Shigella spp., (26.1% from total isolates). The frequency of isolation of Shigella spp., in our study was higher than that of some developing countries (5-10%) (Katouli et al., 1990); however, there are reports from Lao People’s Democratic Republic (16.8%) and Tanzania (12.2%), indicating similar high frequencies (Gascon et al., 2000). The rate of isolation of Shigella spp., in developed countries is about 1% (Essers et al., 2000).

According to the results, the distribution of Shigella spp., in male more than in female represent 54.54 and 45.46% respectively. In this study we found that the incidence of diarrhea in male is almost similar to female. However recent studies showed that no sex difference in the occurrences of diarrhea was found as the risk factors associated with diarrhea are environmental and sociodemographic rather than biological factors (Parashar et al., 1998).

| Age group   | Diarrheal number | Gender |
|-------------|------------------|--------|
|             |                  | Male   | Female |
| 1 month-10 year | 289             | 168    | 121    |
| 10-20 year    | 20               | 13     | 7      |
| 20-30 year    | 19               | 12     | 7      |
| 30-40 year    | 27               | 15     | 12     |
| 40-50 year    | 18               | 10     | 8      |
| 50-60 year    | 18               | 9      | 9      |
| 60-70 year    | 9                | 5      | 4      |
| Total         | 400              | 232    | 168    |

Table 1: General description of gender for patients with acute diarrhea
The first age group (1 month-10 year) showed the high number of isolates (9 isolates), while the lowest incidence were in (10-20 year) and (60-70 year) one isolate to both of them as shown in Table 2. The average age of the patients with Shigella infection in our study was similar to some reports (MoezArdalan et al., 2003), indicating a rise in the average age of Shigella infection to older (aged 12 or more than 12 years) compared to some reports from other developing countries, where the group of one or more than one to less than five year (s) of age had the highest frequency of isolation (Ahmed et al., 1997). While (Rawasheh et al., 1994) noticed that the age group for peak Shigella incidence was 1-4 years.

Shigella flexneri was the most predominant Serogroups (72.7%) followed Shigella boydii (18.2%) and Shigella dysentriae being the least common isolate (9.1%) (Table 3). These results were agreed with the finding (Chuang et al., 2006) they showed that S. flexneri was the most prevalent serotype (73.3%) followed by S. sonnei (26.5%), while the results of some of studies that have been done in Iran, Israel, the United States, Canada and other developed countries. Revealed that S. sonnei is the predominant species in those countries and is more common in children than in adults. However, in Taiwan and Bangladesh the infections are mostly caused by S. flexneri. It has been suggested that factors involved in natural selection may have been the main reason for these discrepancies (Farshad et al., 2006). Though it is in contrast to the finding of developed world but is similar to that in other countries where diarrheal diseases are endemic too (Wasfy et al., 2000).

DISCUSSION

The results of the antibiotic susceptibility tests for four species of Shigella isolates are shown in Table 3. In total, of the 11 isolates, 18.18% (2 isolates) were resistant to Ciprofloxacin, 36.36% (4 isolates) were resistant to Chloramphenicol, 54.54% (6 isolates) were resistant to Cefotaxime, Cefepime, Cefixime and Ceftazidium, 63.63% (7 isolates) were resistant to Azithromycin and 72.72% (8 isolates) were resistant to Tetracycline and Erythromycin. Resistance to Pipracillin, Amoxicillin, Cephalothin, Kanamycin and Rifampicin was 100% detected. These results agree with (MoezArdalan et al., 2003) The sowed the most common resistance among Shigella spp., was to tetracycline (73.5%), trimethoprim-sulphamethoxazole (70.4%) and amoxicillin-clavulanic acid (50.0%). S. flexneri isolates were most frequently resistant to tetracycline (82.2%), amoxicillin-clavulanic acid (82.2%). And disagree with (Banajeh et al., 2001) the noticed most of the Shigella isolates were susceptible to nalidixic acid and cefotaximeand resistant to the other antibiotics. Mandomando et al. (2009) showed that Shigella isolates are resistant mostly to the most available, inexpensive antibiotics by various molecular mechanisms but remain susceptible to ciprofloxacin, which is the first line for empirical treatment of shigellosis in the country.

The results of the antimicrobial susceptibility tests showing a relatively higher number of multidrug-resistant isolates and especially the emergence of resistance to Aminoglycosides and third-generation cephalosporins indicates that designing a surveillance system for antimicrobial resistance in Iraq and the introduction of integrated guidelines for the appropriate use of antibiotics are urgently needed. Multiple resistances with the patterns of Pipracillin, Amoxicillin, Cephalothin, Kanamycin and Rifampicin noticed in all the isolates are shown in Table 4. S. flexneri were showed multiple resistance ranging from 7-13 antibiotic, most of these isolates resistant 12-13 antibiotics.

In the other hand Shigella boydii and Shigella dysentriae showed low resistance pattern they were 6 and 7, respectively. According to the susceptibility of the majority of Shigella spp., to Ciprofloxacin and Chloramphenicol in this study, recommend the more-readily available drug. Chu et al. (1998) indicated multiresistance (resistance to four or more agents) was more common in S. flexneri than in Shigella sonnei. A higher multidrug-resistant rate among Shigella isolates was found in our study. Although only limited numbers of strains were examined, this result may showed a clear trend that multidrug-resistant Shigella in Asian countries has been increasing and this may be due to a worsening situation with regard to antibiotic overuse in both humans and animals (Kuo et al., 2008).

| Age group | Shigella spp., | Male | Female |
|-----------|---------------|------|--------|
| 1 month-10 year | 9 | 5 | 4 |
| 10-20 year | 1 | - | - |
| 20-30 year | - | - | - |
| 30-40 year | - | - | - |
| 40-50 year | - | - | - |
| 50-60 year | - | - | - |
| 60-70 year | 1 | 1 | - |
| Total | 11 | 6(54.54) | 5(45.46) |

| Gender | Shigella flexneri | Shigella boydii | Shigella dysentriae |
|--------|------------------|----------------|-------------------|
| Male   | 4                | 2              | 1                 |
| Female | 4                | -              | -                 |
| Total  | 8                | 2              | 1                 |

Table 2: Distribution Shigella spp., with age group and gender

Table 3: Serogroups of Shigella according to gender
Table 4: Number of antibiotic resistance and plasmid bands of *Shigella* spp.

| Isolate (s) | Antibiotic resistance pattern | No. of antibiotic resistance | No. of plasmid bands |
|-------------|-------------------------------|-----------------------------|---------------------|
| SF1         | CAZ, AZM, K, RA, E, CFM, FEP, CTX, T, KF, AX, PRL | 12                           | 1                   |
| SF2         | CAZ, K, RA, E, CFM, FEP, CTX, T, KF, AX, PRL     | 11                           | 1                   |
| SF3         | CAZ, AZM, K, RA, E, CFM, FEP, CTX, T, KF, AX, PRL | 12                           | 1                   |
| SF4         | CAZ, AZM, K, RA, C, E, CFM, FEP, CTX, T, KF, AX, PRL | 13                           | 2                   |
| SF5         | CAZ, AZM, K, RA, E, CFM, FEP, CTX, T, KF, AX, PRL | 12                           | 1                   |
| SF6         | K, RA, C, E, T, KF, AX, PRL                      | 8                            | 1                   |
| SF7         | K, RA, E, T, KF, AX, PRL                        | 7                            | 1                   |
| SF8         | CAZ, AZM, K, RA, C, E, CFM, FEP, CTX, T, KF, AX, PRL | 13                           | 2                   |
| SB1         | AZM, K, RA, KF, AX, PRL, CIP                    | 7                            | 1                   |
| SB2         | AZM, K, RA, KF, AX, PRL, CIP                    | 7                            | 1                   |
| SD1         | K, RA, C, KF, AX, PRL                          | 6                            | 1                   |

It was found this study there were relation between plasmid bands and multiple antibiotic resistant pattern. As shown in (Table 4). These results agree with Haider et al. (1985) who found that multi-resistant clinical isolates generally harbor a single large transmissible plasmid.

Studies demonstrating the relation of plasmid and drug resistance in clinical isolates of *Shigella* spp., by curing are scanty in our country. Curing of plasmids was carried out with ethidium bromide, sub culture and temperature at 15-30°C. Plasmid curing was achieved by growing the strains, treatment with temperature at 15-30°C and Ethidium bromide, while the lowest curing efficiencies was obtained using subculture as shown in Table 5-7. The plasmid elimination was accompanied by drastic changes in antibiotic resistance and morphology of the colonies (Raja and Selvam, 2009). All isolates those were resistant to ciprofloxacin and chloramphenicol, lose resistance when treated with temperature at 15 and increase inhibition zone diameter when treated with temperature at 30°C while in sub culturing and Ethidium bromide treatment chloramphenicol resistance remained, as well as isolates gave low resistant (inhibition zone) to ciprofloxacin. In another hand third generation cephalosporin affected by both curing treatment temperature at 15-30°C and Ethidium bromide. The results showed isolates death in ethidium bromide at 6.25 concentrations.

After curing experiments the loss of antibiotic resistance was concomitant with the loss of plasmid content so that the results showed that most cured isolates had lost their antibiotic resistance to almost antibiotics tested. This indicates that the resistance determinants of tested antibiotics were located on plasmids. Furthermore, this study suggested that loss of antibiotic resistance phenotype in cured strains may be either because of mutation as a result of incubation in the presence of the curing, or genes encoding resistance to the antibiotics.
### Table 5: Curing of *Shigella* spp., plasmid by heat

| Treatment | Isolate | CIP | C | KF | E | AZM | K | RA | PRL | AX | T | CAZ | CTX | FEP | CFM |
|-----------|---------|----|---|----|---|-----|---|----|-----|----|---|-----|-----|-----|-----|
| BC        |         | 25 | 30| R  | R  | 12 | 13 | R  | R  | R  | R  | 11 | R   | R   | R   |
| H15       | 1       | 31 | 35| R  | R  | 12 | 13 | R  | R  | R  | R  | 11 | 28  | 11  | 25  |
| H30       |         | 35 | 38| R  | R  | 12 | 13 | R  | R  | R  | R  | 11 | 31  | 20  | 32  |
| BC        |         | 22 | 32| R  | 12 | 20 | 12 | 9  | R  | R  | 10 | R   | R   | R   |
| H15       | 2       | 21 | 34| R  | 12 | 19 | 15 | 9  | R  | R  | R  | R   | 11  | R   |
| H30       |         | 21 | 38| R  | 12 | 19 | 16 | 10 | R  | R  | R  | 11 | R   | R   |
| BC        |         | 24 | 30| R  | R  | 11 | R  | R  | R  | R  | R  | R   | R   |
| H15       | 3       | 29 | 31| R  | R  | 13 | R  | R  | R  | R  | 9  | 25  | 14  |
| H30       |         | 31 | 32| R  | R  | 13 | R  | R  | R  | R  | R  | 11 | 29  | 16  |
| BC        |         | 22 | 32| R  | R  | 10 | 12 | 11 | R  | R  | R  | R   | R   |
| H15       | 4       | 22 | 38| R  | 10 | 12 | 13 | R  | R  | R  | R  | R   | R   |
| H30       |         | 22 | 41| R  | 10 | 11 | 16 | R  | R  | R  | R   | R   |
| BC        |         | 22 | 22| R  | R  | 15 | R  | R  | R  | R  | R  | R   | R   |
| H15       | 5       | 22 | 37| R  | R  | 15 | R  | R  | R  | R  | R  | R   | R   |
| H30       |         | 23 | 43| R  | R  | 15 | R  | R  | R  | R  | R   | R   |
| BC        |         | 35 | R  | R  | 22 | R  | R  | R  | R  | 19 | 23  | 23  |
| H15       | 6       | 38 | 22| R  | R  | 22 | R  | R  | R  | R  | 23  | 26  |
| H30       |         | 42 | 25| R  | 23 | R  | R  | R  | R  | R   | 23  |
| BC        |         | 29 | R  | R  | 23 | R  | R  | R  | R  | R   | 22  |
| H15       | 7       | 33 | 21| R  | R  | 23 | R  | R  | R  | R  | 28  |
| H30       |         | 36 | 28| R  | R  | 26 | R  | R  | R  | R   |
| BC        |         | 22 | R  | R  | R  | R  | R  | R  | R  | R   |
| H15       | 8       | 26 | 20| R  | R  | R  | R  | R  | R  | R   |
| H30       |         | 27 | 22| R  | R  | 12 | 9  | R  | R  | R   |
| BC        |         | 24 | 30| R  | R  | R  | R  | R  | R   |
| H15       | 9       | 22 | 33| R  | R  | 19 | R  | R  | R   |
| H30       |         | 27 | 38| R  | R  | 19 | R  | R  | R   |
| BC        |         | 29 | R  | R  | 20 | R  | R  | R   |
| H15       | 10      | 23 | 34| R  | R  | R  | R  | R   |
| H30       |         | 26 | 38| R  | R  | 22 | R  | R  | R   |
| BC        |         | 30 | R  | R  | 23 | R  | R  | R   |
| H15       | 11      | 38 | 22| R  | R  | 23 | R  | R  | R   |
| H30       |         | 41 | 28| R  | R  | 23 | R  | R  | R   |

### Table 6: Curing of *Shigella* spp., plasmid by sub culture

| Treatment | Isolate | CIP | C | KF | E | AZM | K | RA | PRL | AX | T | CAZ | CTX | FEP | CFM |
|-----------|---------|----|---|----|---|-----|---|----|-----|----|---|-----|-----|-----|-----|
| BC        |         | 25 | 30| R  | R  | 12 | 13 | R  | R  | R  | R  | 11 | R   | R   |
| SC        |         | 25 | 30| R  | 11 | 27 | 20 | R  | R  | R  | R   | 20  |
| BC        |         | 22 | 32| R  | 12 | 20 | 12 | 9  | R  | R  | R   |
| SC        |         | 32 | 32| R  | 12 | 25 | 21 | R  | R  | R   |
| BC        |         | 24 | 30| R  | R  | 11 | R  | R  | R   |
| SC        |         | 30 | 30| R  | R  | 11 | R  | R  |
| BC        |         | 22 | 21| R  | 10 | 12 | 11 | R  | R  |
| SC        |         | 33 | 21| R  | 11 | 12 | 11 | R  | R   |
| BC        |         | 22 | 22| R  | R  | 15 | R  | R  |
| SC        |         | 31 | 22| R  | R  | 15 | R  | R  |
| BC        |         | 35 | R  | R  | 22 | R  | R  | R  |
| SC        |         | 35 | R  | 10 | 27 | 19 | R  | R  |
| BC        |         | 29 | R  | R  | 23 | R  | R  | R  |
| SC        |         | 29 | R  | R  | 11 | 24 | 20 | R  | R  |
| BC        |         | 22 | R  | R  | R  | R  | R  |
| SC        |         | 26 | R  | R  | 10 | 27 | 19 | R  |
| BC        |         | 9  | R  | 29 | R  | 19 | R  | R   |
| SC        |         | 12 | 20| R  | R  | 21 | R  | R  |
| BC        |         | 10 | R  | 29 | R  | 20 | R  | R  |
| SC        |         | 13 | 30| R  | R  | 20 | R  | R  |
| BC        |         | 11 | 30| R  | R  | 23 | R  | R  |
| SC        |         | 30 | R  | R  | 23 | 27 | 18 | R  |

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Table 7: Curing of Shigella spp., plasmid by ethidium bromide

| Treatment | Isolate | CIP | C | KF | E | AZM | K | RA | PRL | AX | T | CAZ | CTX | FEP | CFM |
|-----------|---------|----|---|---|---|-----|---|----|-----|----|---|-----|------|-----|-----|
| BC        | 25      | 30 | R | R | R | 12  | 13 | R  | R   | R  | R  | R   | 11   | R   | R   |
| E31.25    | 1       | 29 | 32 | R | R | 24  | 20 | R  | R   | R  | R  | 15   | 26   | 15  | 16  |
| E62.5     | 29      | 37 | R | R | R | 24  | 21 | R  | R   | R  | R  | 15   | 26   | 15  | 16  |
| BC        | 22      | 32 | R | R | 12  | 20  | 12 | 9  | R   | R  | R  | 10   | 15   | R   | R   |
| E31.25    | 2       | 22 | 33 | R | 13 | 22  | 12 | 10 | R   | R   | 10  | 9    | 8    | R    |     |
| E62.5     | /       | /  | /  | /  | /  | /    | /  | /  | /    | /  | /  | /    | /    | /   | /   |
| BC        | 24      | 30 | R | R | 24  | 11  | 12 | 9  | R   | R   | R  | 15   | 30   | 42  | 22  |
| E31.25    | 43      | 40 | R | R | 31  | 25  | 5  | 5  | 5   | 30  | 42  | 22   | 25   |     |     |
| E62.5     | /       | /  | /  | /  | /  | /    | /  | /  | /    | /  | /  | /    | /    | /   | /   |
| BC        | 22      | 10 | 12 | 11 | R  | 10   | R  | R  | R   | R  | R  | R    |     |     |     |
| E31.25    | 4       | 30 | R | R | 10  | 24  | 19 | R  | R   | R  | 17  | 15   | 25   | 14  | 16  |
| E62.5     | /       | /  | /  | /  | /  | /    | /  | /  | /    | /  | /  | /    | /    | /   | /   |
| BC        | 22      | 22 | R | R | R  | 15   | R  | R  | 15   | R  | R  | R    |     |     |     |
| E31.25    | 5       | 30 | 24 | R | R | 15   | R  | R  | R   | R  | R  | R    |     |     |     |
| E62.5     | /       | /  | /  | /  | /  | /    | /  | /  | /    | /  | /  | /    | /    | /   | /   |
| BC        | 35      | R  | R | R  | 22  | R  | R  | R   | 19  | 23  | 23   | 19   |     |     |
| E31.25    | 4       | 44 | R | R | 23  | R  | R  | R   | 20  | 23  | 24   | 22   |     |     |
| E62.5     | /       | /  | /  | /  | /  | /    | /  | /  | /    | /  | /  | /    | /    | /   | /   |
| BC        | 29      | R  | R | R  | 23   | R  | R  | R   | 22  | 26  | 19   | 22   |     |     |
| E31.25    | 7       | 30 | R  | R | R  | 22  | 19  | R  | R   | R  | 22  | 26   | 20   | 21   |     |
| E62.5     | 30      | R  | R | R  | 22  | 21  | R  | R  | R   | 23  | 27  | 21   | 22   |     |     |
| BC        | 22      | R  | R | R  | 22  | 21  | R  | R  | R   | R  | R  | R    |     |     |     |
| E31.25    | 8       | 28 | R  | R | R  | 24  | 18  | R  | R   | R  | 16  | 25   | 14   | 15   |     |
| E62.5     | /       | /  | /  | /  | /  | /    | /  | /  | /    | /  | /  | /    | /    | /   | /   |
| BC        | R       | 29 | R  | R | 19  | R  | R  | R   | 23  | 26  | 25   | 25   |     |     |
| E31.25    | 9       | 19 | 30 | R | R  | 19   | R  | R  | 24  | 26  | 25   |     |     |     |
| E62.5     | /       | /  | /  | /  | /  | /    | /  | /  | /    | /  | /  | /    | /    | /   | /   |
| BC        | R       | 29 | R  | R | 20  | R  | R  | R   | 22  | 28  | 25   | 22   |     |     |
| E31.25    | 10      | 17 | 30 | R | R  | 20  | R  | R  | R   | 25  | 26  | 27   | 26   |     |     |
| E62.5     | /       | /  | /  | /  | /  | /    | /  | /  | /    | /  | /  | /    | /    | /   | /   |
| BC        | 30      | R  | R | 23  | 21  | R  | R  | R   | 24  | 28  | 26   | 23   |     |     |
| E31.25    | 11      | 33 | R  | R | 22  | 21  | R  | R  | R   | 24  | 28  | 23   | 26   |     |     |
| E62.5     | 34      | R  | R | 23  | 21  | R  | R  | R   | 24  | 28  | 26   | 23   |     |     |

CONCLUSION

Approximately 89.5% of the diarrhea cases had no bacterial pathogen, suggested of probability of viral infection. Similar to other studies (Patel et al., 2008) which indicated that 84% of diarrheal patients caused by other microorganism than other bacteria.

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