

Full Length Research Paper

Bacterial pathogens associated with infectious diarrhea in King Khalid University Hospital, Riyadh Saudi Arabia 2005-2010

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Diarrhoeal diseases are major cause of morbidity worldwide. In developed and developing countries, *Salmonella*, *Shigella* and *Campylobacter* species are the main bacterial causes. This study aimed to investigate the occurrence and antimicrobial susceptibility of bacteria causing diarrhea in Riyadh, during a six years period. This study was performed in Riyadh capitol of Saudi Arabia between January 2005 to December 2010. The data was obtained from request forms for stool examination of patients complaining of diarrhea. Specimens were cultured; isolates were identified biochemically and serologically following conventional methods. Two percent of specimens tested were positive, 89.1% of these grew *salmonella* species, 5.6% *Shigella* species and 5.3% grew *campylobacter jejuni*. Most of these isolates were from the age group >1-5 years old. Cefotaxime was the most active agent against *salmonella* and *shigella*, while erythromycin was the most active agent against the *C. jejuni*. *Salmonella*, *Shigella* and *Campylobacter* species were the main isolates from stool of patient with diarrhoea. Cefotaxime was the most active agent on *Salmonella* and *shigella* species, while erythromycin was the most active agent against *C. jejuni*.

Key words: Diarrhea, *Salmonella*, *Shigella campylobacter*, Saudi Arabia.

INTRODUCTION

Diarrhoeal diseases are one of the five leading causes of death worldwide (Wanke et al., 2013a). Globally, one in ten children deaths results from diarrhoeal diseases during the first 5 years of life which results in 800,000 fatalities worldwide annually; most of these occurring in subsharan countries (Kotloff et al., 2013). In developed countries (USA) acute infectious diarrhea results in more

than 1.5 million patient visits to doctors and 200,000 hospitalization annually (Kotloff et al., 2013). In developed countries viruses predominate as main causes of diarrhea but the main bacterial causes of diarrhea include *Campylobacter*, *Salmonella*, *Shigella* species, *Aeromonas* species and *Escherichia coli* (Wanke et al., 2013b). However sporadic cases of diarrhea due to vibrio

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Table 1. Shows the total number of specimens tested, number and percentage of negative and positives specimen for pathogens in each of the six years of the study.

Parameter	2005	2006	2007	2008	2009	2010	Total
Positive (%)	114 (3)	98 (3)	99 (2.9)	104 (2.5)	62 (1.4)	74 (1.8)	551 (2.4)
Negative	3480	3399	3262	4041	4292	4390	22,864
Total	3587	3303	3365	4142	4378	4140	22,915

cholerae and Yersina do occur in these countries. The types of etiological bacterial agents of diarrhea in developing countries are similar to those in developed countries; however *Shigella*, *Salmonella* and vibrios predominate more in developing countries (Kumar and Subitha, 2012).

The aim of this study was to investigate the role of bacterial pathogens in cases of diarrhea in a major teaching hospital Riyadh the capital of the Kingdom of Saudi Arabia during a six year period. The study also aimed to see the antimicrobial resistance of these bacterial pathogens and compare it to previous studies in the area of the study, the kingdom of Saudi Arabia as a whole and some worldwide studies.

King Khalid University Hospital (KKUH) is the main teaching hospital in the Kingdom of Saudi Arabia with about 900 bed capacity. It is a tertiary care hospital serving a population of 2-3 millions of local and expatriates nationals. To the best of our knowledge, only few such studies were done in recent years from this area. The rapid worldwide development of bacterial resistance to antimicrobial agents necessitates such studies to follow up these changes.

MATERIALS AND METHODS

Laboratory request forms for stool specimens' examination were used to retrospectively get data related to patients from whom these specimens were collected during the period of January 2005 to December of 2010. Data collected included demographic data of patients, documented clinical features, identification and antimicrobial susceptibility of bacterial pathogens isolated.

Laboratory testing

Stool specimens were processed by culturing directly onto MacConkey agar, XLD agar plates and salenite F enrichment broth. The latter medium was sub-cultured onto MacConkey and Hektoen agars after overnight incubation at 35°C.

All plates were examined for *Salmonella* species, *Shigella* species and *E. coli* by standard microbiological methods. The MicroScan automatic system (Dade Behring a Siemens Company) was used for biochemical identification. Commercial anti-sera were used for fine focused serological identification of the isolates (Difco laboratories detroit, Mich USA).

For detection of *Campylobacter jejuni* and *Campylobacter* species, stools were cultured on Campy plate selective medium incubated at 42°C for 48 h under microaerophilic conditions. Identification of *C. jejuni* was done by the conventional methods

including gram stain, oxidase test, hippurate utilization and nalidixic acid susceptibility. The *Campylobacter* isolates were identified as *C. jejuni* or otherwise as *Campylobacter* species.

Laboratory processing of faecal specimens for *Vibrio* species and *E. coli* (0157 H7) as well as other rarely occurring pathogens was done if requested for or indicated by clinical features. This was done by inoculating faecal specimens on Thiosulfate-citrate bile salts (TCBS) for vibrios and sorbitol MacConkey for *E. coli* (0157 H7) respectively.

Detection of *Yersinia enterocolitica* is done by cold temperature enrichment procedure by incubating phosphate buffered saline incubated at 4°C for three weeks.

For detection of *Aeromonas* species group of organisms, stool samples were plated on sheep blood with incorporation of ampicillin (30 µg/ml) agar incubated at 35°C for 18 to 24 h. Presumptive haemolytic *Aeromonas* colonies if found were picked and tested for oxidase production. All oxidase positive colonies were further identified by the MicroScan automated system.

RESULTS

From January 2005 to December 2010 a total of 22,311 stool specimens were submitted for culture, 551 specimens were positive for some enteric bacterial pathogens, indicating 2% positivity. Of these *salmonella* species were the commonest bacterial pathogens isolated with a number of 491 shown (89.1%) of the total positive isolates followed by *C. jejuni* with a number of 29 and a percentage of 5.3% then *Shigella* species accounting for 31 isolates and 5.6% of the total positives. No *Aeromonas* species, *Yersinia enterocolitica* vibrios or *E. coli* 0157 H7 were isolated in this study.

Table 1 shows the numbers and percentages of the total negative and positive specimens from the total specimens tested. The ranges of the positive specimen numbers ranges between 114 in the year 2005 and 62 in the year 2009. However the percentage of positivity of specimen each year does not differ much in different years, it range between (3 and 1.4%) in the years 2005 and 2009, respectively.

Table 2 shows the number of patients in different age groups from whom the enteric bacterial pathogens were isolated. The age group >1-5 years was the most affected age range with a number of 194 patients (35.2%) having positive results, followed by the >15-50 age group represented by 187 patients (33.9%) with positive results. The age group <1 year of age was the least affected age group showing 3 patients (0.5%) with positive results.

Table 3 shows the number and percentages of

Table 2. Number and percentage of patients in different age groups from whom the enteric bacterial pathogens were isolated during the six year period of the study (No. 551).

Age (years)	Number	Percentage
<1	3	0.5
1-5	194	35.2
>5-15	116	21.1
>15-50	187	33.9
>50	51	9.3
Total	551	100

Table 3. Shows the numbers and percentage of patients nationality from whom the enteric pathogen were isolates No. (551).

Nationality	Frequency	Percentage
Saudi	427	78.3
Indian	24	4.4
Filipino	20	3.7
Pakistan	14	2.6
Egyptian	13	2.4
Sudanese	9	1.7
Jordania	8	1.5
Indonesia	7	1.3
Yemeni	7	1.3
Unknown	6	1.1
Syrian	5	0.9
Banglade	4	0.7
Algerian	1	0.2
Eriteria	1	0.2
Iraqi	1	0.2
Lebanese	1	0.2
Nepali	1	0.2
Srilanka	1	0.2
Tanzania	1	0.2
Total	551	100

nationality of patients from whom the enteric pathogens were isolated. The majority of the patients with positive stool culture were Saudi 427 (78.3%), followed by Indian 24 (4.4%) and Filipinos 20 (3.7%). Male gender was predominant as it is represented by 322 patients (58.4%) versus female showing 229 patients (41.6%). The different nationalities have different habits and different accommodation facilities.

Figure 1 shows the percentage of resistance of *C. jejuni* isolates to antimicrobial agents tested. The highest resistance was to cephradine (96%) followed by nalidixic acid (79%). However erythromycin was the most active agent against these isolates.

Figure 2 shows the percentage of resistance of *shigella*

species isolates tested to the antimicrobial agents used. The highest activity against these isolates was shown by cefotaxime which shows 0% resistance, followed by ciprofloxacin showing 6.7% resistance while the highest resistance was shown to ampicillin (53.30%).

Figure 3 shows the percentage of resistance of *salmonella* species isolates to antimicrobial agents. The highest activity was shown by cefotaxime with 2.10% resistance followed by chloramphenicol with 8.20% resistance. However the highest resistance was shown to nalidixic acid (43.30%) and ciprofloxacin resistance to (34.30%) and then cephalothin with (33.30%) resistance.

DISCUSSION

Diarrheal diseases have great impact on human health especially on children (Kosek et al., 2003). A study comparing this burden between the periods of 1954-1979 and the period of 1990-1989 showed a decline of mortality in children under 5 from an estimate of 13.6 to 5.6 to 4.9/1000 per year (Kosek et al., 2003). Diarrheal diseases are major causes of morbidity with attack rates ranging from 2 to 12 per person per year in developed and developing countries (Glass et al., 1996). The causes of diarrhoea include a wide range of virus (Glass et al., 1996; Guerrant et al., 1990) like rota virus (Tucker et al., 1998), bacteria (Mastson and Estes, 1990) and parasites (Piekering, 1990).

This retrospective study of ours was based on information from laboratory reports. Accordingly the information on clinical features of the patients may not be complete. However important facts derived from this study are the rate of isolation and antimicrobial susceptibility of bacterial gastrointestinal pathogens. It clearly reflects the importance of *Salmonella* species as cause of diarrhoea in this part of the world as documented by other studies (Kosek et al., 2003). The antimicrobial susceptibility of these isolates in our study showed high resistance to ampicillin a result that concurs with a previous studies from our institution (Somily et al., 2012) and others (Holmes et al., 1996) but differ from other (Avendaño et al., 1993; Hardy et al., 1994). This study of ours also shows the importance of *C. jejuni* as a cause of diarrhea in this region as has been reported in developed and developing countries (Guerrant et al., 1999). Our low results on *shigella* species isolates are similar to those from developed countries but differ from those reported from developing countries where *Shigella* species are not an uncommon cause of diarrhea (Koopman et al., 1984). These results rank the Kingdom of Saudi Arabia with, its well-developed water and drainage system at least in the area of our study, between the developing and developed countries.

During this period of the study no *Clostridium difficile*, *Yersinia*, *Vibrio*, *E. coli* O157 H7, *Aeromonas* or *Pleisiomonas* species were isolated. These pathogens are either rare in our area or other parts of the Kingdom

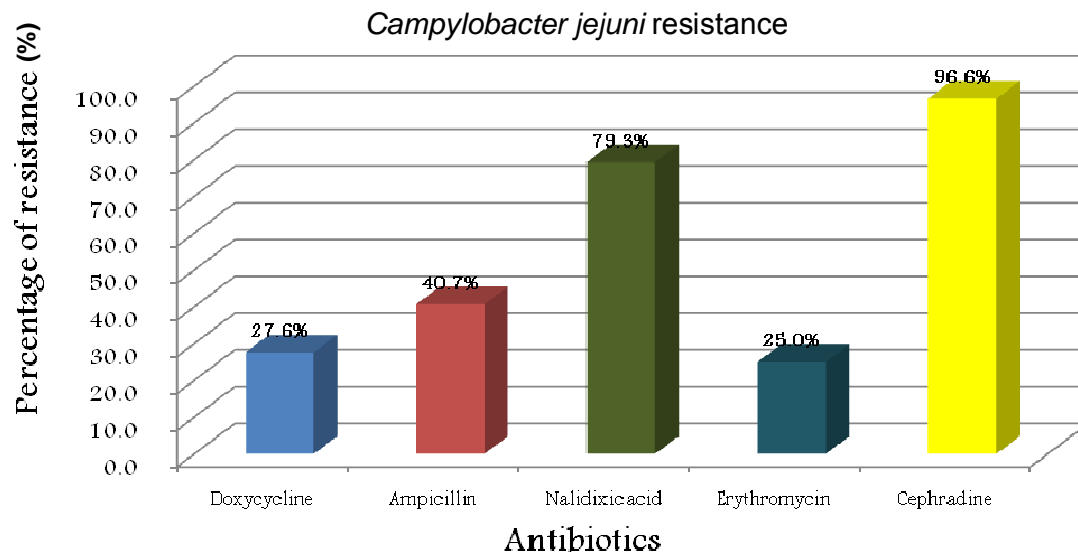


Figure 1. Percentage of resistance of *Campylobacter jejuni* to commonly used antibiotics (No.78).

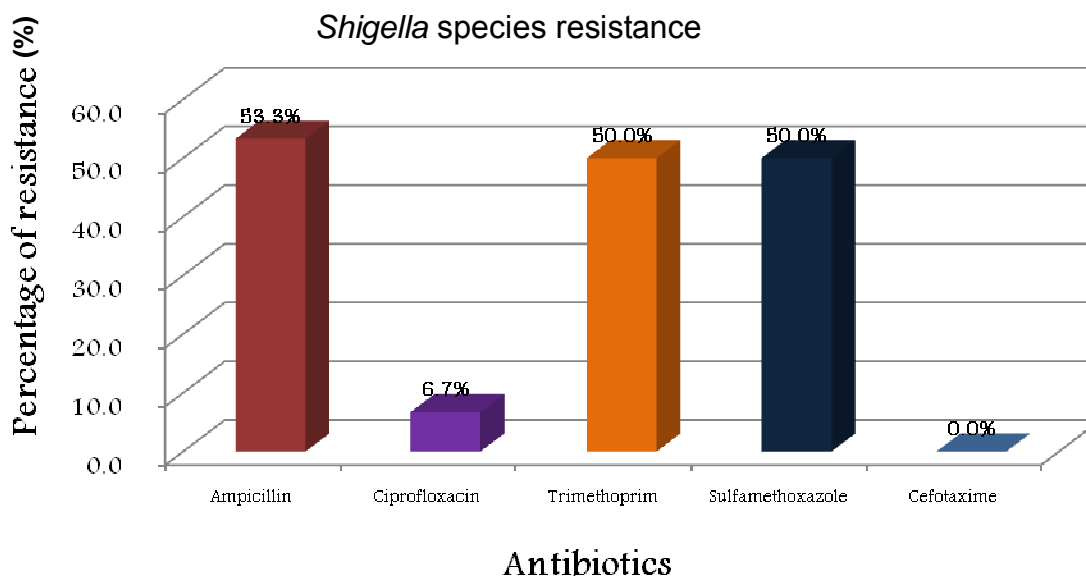


Figure 2. Percentage of resistance of *Shigella* species to commonly used antibiotics (No.46).

(CDC, 2003). They may be unexpected or not looked for in the kingdom as a whole, like other pathogens. However they may only be looked for during the Hajj season or places where people from different nationalities attend this occasion. However more investigation is needed in this aspect to look for these rarely isolated pathogens (Dennehy, 2005; Albert et al., 1999). An explanation for the reduction of the rate of the isolation of enteric pathogens in our study may be due to the high selectivity of our stools culture media. As recommended by CLSI we advise that the quality and

selectivity of these media should be checked using ATCC organisms to test the performance and selectivity of enteric media before use (CLSI, 2004).

Shigella species known as common cause of bacterial gastroenteritis after *Salmonella* in developed and developing countries (Koopman et al., 1984; CDC, 2003) was detected in only a small number of our cases (Mikhail, et al., 1990).

C. jejuni has been recognized as the most common cause of sporadic bacterial gastroenteritis in many countries (Sojo et al., 1982; Blaser et al., 1982). Again

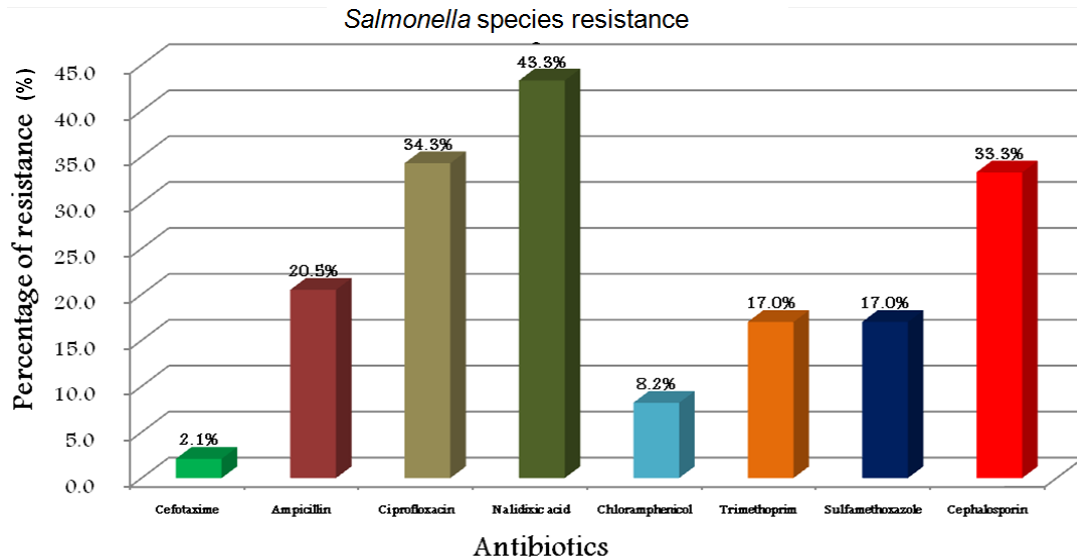


Figure 3. Percentage of resistance of *Salmonella* species to commonly used antibiotics (No.427).

the fastidious nature of this organism and absence of quality control (QC) of *Campylobacter* media raises the possibility of under detection of these pathogens in our study (CLSI, 2010).

In our study around 80% of *C. jejuni* were resistant to Nalidixic acid which is a surrogate that indicates ciprofloxacin resistance and this coincides with recent literature of fluoroquinolone resistance. This may be due to the use of these agents in poultry and in veterinary medicine in general, which has increased the reservoir of resistant *Campylobacter* species (Smith et al., 1999) although ciprofloxacin was not tested on *Campylobacter* during this study we concluded this from resistance to Nalidixic acid.

Resistance of *C. jejuni* to erythromycin in this study was 70% which may result from mutations in the genes encoding ribosomal proteins or it may also result as has been claimed before to be associated with decreased permeability of the cell envelope as in enterobacteriaceae, including plasmid mediated mechanism (Burnens et al., 1996; Steinbigel, 1990).

Our results on *C. jejuni* resistance to macrolides is consistent with other studies as it has been previously noticed that the incidence of macrolide resistance among *C. jejuni* and *Campylobacter coli* is highly variable with respect to the origin country of isolation. It is lowest in Canada (12%) and Germany (0%) (*C. jejuni*) (Gibreel et al., 2005; Gaudreau and Gilbert, 2003; Lubber et al., 2003) and highest in Taiwan (*C. coli*) 50% (Li et al., 1998) and Nigeria 79.8% (Smith et al., 1999). However a study on macrolide resistance in *C. jejuni* in Sweden showed stable pattern (Engberg et al., 2001; Osterlund et al., 2003).

The high rate of resistance of macrolide in *campylobacter* species may be related to the extensive

veterinary use of macrolides (Moore et al., 1996; Ishihara et al., 2004) which is more prominent in *C. coli* than *C. jejuni* as in Japan all *C. jejuni* were susceptible to macrolide and 48.4% of *C. coli* were resistant (Ishihara et al., 2004). As there is study from our region in relation of antimicrobial in no veterinary medical. It is difficult to reach a conclusion concerning the effect of this as resistance of *campylobacter* species in our study.

Conclusion

Salmonella species, *Shigella* species and *C. jejuni* are the most common enteric pathogens isolated from patients complaining of diarrhea in a teaching hospital in Riyadh Saudi Arabia. Pathogen like, *E. coli* 0157 H7 vibrios, *Aeromonas*, *Plesiomonas* were not encountered in this study.

Resistance of *Salmonella*, *Shigella* and *Campylobacter* concur with different results from developing countries. Media used for culture of stool should be quality controlled to make sure that some enteric pathogens are not missed or overlooked.

Conflict of Interests

The author(s) have not declared any conflict of interests.

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