INTRODUCTION
Antibiotics with unique properties have been developed during the last 50 years, with antibiotics being regarded as the specific and targeted therapy for most of the infectious diseases. As newer antimicrobials come into existence, there are two major problems associated with these drugs, especially in third world/developing countries via drug resistance and adverse effects. One of the commonest side effects associated with the majority of commercially available antibiotics is diarrhoea, noted most often 3 – 7 days after starting therapy. Virtually all antimicrobials can cause diarrhoea of variable severity. The cause of this diarrhoea is most probably the alteration of normal bacterial flora of the intestines, which disrupts the milieu interior of the gut and allows the opportunistic microorganisms to grow and cause loose motions. The implicating agent has been debated for the last 50 years with the first organism suggested for postantibiotic diarrhoea being *Staphylococcus aureus*. Later, anaerobic coliform were suggested, but finally in 1978, most of the workers agreed that the majority of cases involving postantibiotic diarrhoea were related with powerful exotoxins released by *Clostridium difficile*. Due to the non-enforcement of laws regarding antibiotic prescription and sales, problems related to ‘drug resistance’ against even the most common microbiological strains such as *Salmonella typhi* are being reported as well as the dangerous complications like pseudomembranous colitis caused by *Clostridium difficile*. The aim of this study was to determine the frequency of *Clostridium difficile* in postantibiotic diarrhoea among admitted patients in medical ward of a secondary care hospital of Karachi as well as to implicate the antibiotics responsible for this diarrhoea.

METHODOLOGY
It was a single centre observational study conducted at Sindh Government Hospital, Liaquatabad, Karachi, between June 2002 and May 2009 for a total duration of 7 years. The patients who were admitted to the medical ward for different reasons and who developed diarrhoea after atleast 2 days of taking antibiotics via oral or parenteral routes, with fecal leukocytosis were selected.
for this study. The exclusion criteria were patients with a history of inflammatory bowel disease or malabsorption states and patients who had no leukocytes in stools, and had formed stools.

The selected patients' information was collected by the trained resident medical officers of the respective medical unit with verbal consent being taken from patients before further proceedings. A data collection form was designed and all essential variables were recorded including age, gender, address, occupation, presenting complaints with duration, use of antibiotics with dose, incubation period of diarrhoea, number and types of stools, past medical history, educational status and addictions. Three samples of stool for each patient were collected in the bottles provided by the laboratory and sent for detailed report including microscopy for WBC (white blood cells) and for culture on cycloserine - cefotoxine, fructose - agar (CCFA) medium. The culture samples were processed and analyzed by qualified experimental microbiologists. All stool reports were collected after one week and entered in a register.

Data analysis was performed with statistical software SPSS - 16 (SPSS, Chicago, USA) and expressed as mean values with standard deviation and 95% confidence interval.

RESULTS
A total of 1,680 patients were admitted to the medical ward for various indications over the course of 7 years. Of these, 473 patients (28.15%) received antibiotics, and 191 patients (40.38%) developed postantibiotic diarrhoea. The mean age of patients suffering from antibiotic associated diarrhoea (AAD) was 45.66 ± 17.73 years (Figure 1). The male to female ratio was 1.85:1. All the patients admitted for AAD were from Liaquatabad town, Karachi. The mean incubation period of AAD was 3.16 ± 0.67 days. The mean frequency of watery stools was 4.84 ± 1.24/day. The mean leukocyte/HPF in stool DR was 6.67 ± 0.89. The stool culture on CCFA medium was positive in 57 patients (29.18%), and they were labelled as Clostridium difficile associated diarrhoea" (CDAD), while 134 patients (70.15%) were negative and labelled as benign antibiotic associated diarrhoea" (BAAD). Ampicillin and amoxicillin were most commonly implicated antibiotics (Figure 2).

DISCUSSION
Clostridium (C.) difficile is a treatable pathogen and is an important cause of nosocomial and iatrogenic disease. Consequently it is important to be able to establish its diagnosis as quickly as possible. In a country with very limited resources, and especially in primary and secondary care settings where facilities are limited, we tried to address this important issue. In this study of hospitalized patients, the association of C. difficile was found to be 29.18%, which is near to the study done by Yablon et al. in 1993 who detected a 25% association of C. difficile.10 The mean age of involvement was found to be 45.66 years which is comparable to the study done by Mannabe and his co-workers in 1995, who found it to be 52.80 years.11 The difference is not wide and showed that CDAD usually does not involve the younger age groups.

Although cytotoxin assays, latex agglutination test, ELISA and PCR of the stools are the latest and least time consuming investigations for the diagnosis of CDAD,12 fecal leukocytes detection in the stools was used as the first step to narrow the diagnosis, and then culture on CCFA to confirm it.13,14 The above named...
tests are still not widely available and are highly unaffordable by the majority of the patients. The catchment area for the study centre belonged to a low-income class. On the other hand, stool culture on CCFA is comparatively easy to perform and much more cost-effective with chances of reported error being less in expert hands. Gerding and colleagues stated that stool culture has been a mainstay in laboratory diagnosis of CDAD. Comparing the cytotoxin assays whose sensitivity is 67 – 100% and specificity is 85 – 100%, the stool culture sensitivity is 89-100% and specificity is 67 – 100%, the difference is not marked considering the availability and cost effectiveness. In this study, 134 patients (70.15%) showed leukocytes in stools but were negative for stool culture. Stool DR was also negative for any parasite; these were labelled as benign antibiotic associated diarrhoea (BAAD). In these patients, the diarrhoea probably occurred due to the pharmacokinetics of the antibiotics or direct irritant effect of drugs on intestinal mucosa; these facts are supported by Bartlett.

In most of the public hospitals, the usual supply of antibiotics to the wards are benzylpenicillin, ampicillin and gentamicin as they cover most of the gram positive and negative organisms. The record of Sindh Government Hospital, Liaquatabad, Karachi, showed that the antibiotics that had been supplied from stores to medical ward were ampicillin, amoxicillin with clavulanate, gentamicin, cefoxitin, ciprofloxacin, clarithromycin and azithromycin. In comparison, leading antibiotics being used in the foreign countries are the third and fourth generation cephalosporins, quinolones, macrolides and new broad spectrum penicillins. This means that AAD in developed countries may have different aetiologies than in our settings as we are still using economical and older antimicrobials in the public sector.

However, comparing the present results of antibiotics that caused CDAD with Delmee and Avisani in 1988, the commonest groups of antibiotics were ampicillin, comprising of 35.08% of all antibiotics in our settings, whereas the other study showed the commonest antibiotic was cephalosporin which comprised 32.11%. The other groups of antibiotic results are shown in Table I. This comparison inferred that macrolides and quinolones are comparatively safer agent to use in relation with CDAD.

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>Delmee and Avisani (%)</th>
<th>Present study (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inj. Ampicillin</td>
<td>7.81</td>
<td>35.08</td>
</tr>
<tr>
<td>Inj. Cefoxitin</td>
<td>32.11</td>
<td>15.14</td>
</tr>
<tr>
<td>Macrolides (oral)</td>
<td>1.5</td>
<td>17.91</td>
</tr>
<tr>
<td>Ampicillin with Gentamicin</td>
<td>7.81</td>
<td>4.71</td>
</tr>
<tr>
<td>Quinolones (oral)</td>
<td>3.12</td>
<td>8.90</td>
</tr>
</tbody>
</table>

This study was performed at one secondary care hospital, and it will be more informative if it is further continued in multiple centres involving the primary care settings. The other limitation is the non-availability of cytotoxin assays which can broaden the spectrum of CDAD.

CONCLUSION

The diagnosis of antibiotics associated diarrhea (AAD) can be done with stool cultures by experienced microbiologists when cytotoxin assays are not available. The majority of cases of AAD were stool culture negative for Clostridium difficile. These cases were self-limiting. Those who were stool culture positive showed that the major offending antibiotics were ampicillin/amoxicillin. Macrolides and quinolones are still safer drugs to use in order to avoid the consequence of CDAD.

REFERENCES