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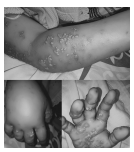

8 year F, with recurrent infections. Hospitalized to us once, however required multiple antibiotic courses for any year. She is failure to thrive. Recently she was admitted with generalized and dermatomal vesicular lesions on arms, hands and body and was treated at Larkana. Father has other routine investigations done and were found to be normal. Her more specific labs are below. Her HIV was negative from Larkana
Flow cytometry showed CD3+ total T-lymphocytosis. CD4+ T-helper lymphocytes are severely low with CD8+ T-regulatory lymphocytosis. CD4+/CD8+ ratio is reversed. CD19+ total B-lymphocytes are low. Immunoglobulin levels showed IgA 3.58 (0.4-3.5); IgM 2.42 (0.5-3); IgG 52.48 (6.5-16); IgE 8.11
Her repeat HIV assay, 4th Generation Elisa and HIV 1 confirmation assay came out positive.

Courtesy : Ali Faisal Saleem, Assistant professor, Paediatric Infectious Diseases, Aga Khan University, Karachi, Pakistan

XDR Typhoid Outbreak in Hyderabad – Tools for Containment and Management

Typhoid remains a serious health issue in middle and lower middle income countries of Africa and South Asia. More than 90% of enteric fever-related morbidity and mortality is reported from Asia, and studies from some Asian countries have shown that incidence of typhoid fever is highest among children aged <15 years.[1,2] In addition to the high disease burden, multi-drug antimicrobial resistance in *Salmonella Typhi (S. typhi)* is a growing threat. Strains resistant to chloramphenicol, trimethoprim-sulfamethoxazole, and ampicillin (Multi Drug Resistant; MDR) are endemic in many Asian and sub-Saharan African countries. Pakistan has high rates of both multidrug resistant typhoid (60%) and fluoroquinolones resistant (>90%) *S.typhi* infection however resistance to 3rd generation cephalosporin remained less than 1% until November 2016.[3,4] The Extensively drug resistant (XDR) *S. typhi* is resistant to 5 classes of antibiotics including (chloramphenicol, trimethoprim-sulfamethoxole, ampicillin, flour quinolones and Cephalosporin). XDR typhoid was first identified in Hyderabad city of Sindh province in November 2016. The outbreak has now spread to other cities in Sindh particularly large numbers are being reported from Karachi. To date, more than 2,000 cases of XDR typhoid have been reported in Karachi, and there may be more, as typhoid is often underestimated due to few people with typhoid having their blood cultured, the 60% sensitivity of this diagnostic test and known underreporting. Testing of drinking water samples from the affected communities revealed faecal contamination in more than 60% of the water samples. Similarly, *S. typhi* DNA was detected in some of the drinking water samples.

In response to this outbreak, a mass immunization campaign using typhoid conjugate vaccine (TCV) was initiated in Hyderabad, Pakistan. The age distribution of cases was important in the context of the choice of the intervention strategy to control the outbreak. Historically, typhoid vaccines (Vi polysaccharide) have had only modest efficacy, a short duration of protection, and could not be administered to children <2 years of age. However, TCV has shown good immunogenicity and can safely be given to children =6 months of age and was recently prequalified by WHO.[5] We determined that a wide-scale TCV campaign offered the most cost-effective and rapid approach to interrupting transmission of this dangerous newly emerged XDR *S.Typhi*. This is the first example of vaccination at a mass scale being implemented for the control of an outbreak of extremely drug resistant typhoid. The number of cases of typhoid in Hyderabad has significantly decreased since the start of the vaccination. Mass immunization for the control of this outbreak in Hyderabad led to the approval of typhoid conjugate vaccine in routine immunization in Pakistan and will be introduced in 2019.

Health education and awareness activities were also initiated with the help of community health workers. Pamphlets were developed and translated in local languages Sindhi and Urdu and circulated door-to-door with the help of community mobilizers and community health workers. In addition, we also educated the polio workers and provided them these pamphlets and handbills to distribute during polio campaigns. Messages on hand hygiene, water boiling, cleaning of raw vegetables/fruits and personal hygiene were delivered door-to-door during the polio immunization campaigns.

The high number of cases and the challenge of limited therapeutic options required an immediate response to limit the damage and prevent the further spread of XDR typhoid. Continuing medical education (CME) sessions were organized for the paediatricians and general practitioners of Hyderabad to inform them about the XDR typhoid outbreak, operational definitions to identify suspected typhoid cases were developed and guidelines for treating XDR typhoid cases were shared. Strong emphasis was laid on confirmation of diagnosis using blood culture and sensitivity and serologic tests such as typhidot were discouraged at all platforms.

To conclude, the lessons and experiences gained during the outbreak investigation and control interventions in Hyderabad provide a useful roadmap for the scaling-up of typhoid control strategies in Karachi and other cities.

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ORIGINAL ARTICLE

Frequency Distribution of Bacterial Vaginosis Using Nugent Score and Culture in Women with Vaginal Discharge

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Abstract

Introduction
Accurate and timely identification of *Bacterial vaginosis* (BV) from high vaginal swab of high-risk patients with vaginal discharge has been found to be a cost effective measure for early diagnosis and treatment of patient, it also decreases the burden of health care cost and complications

Objectives
To determine the frequency distribution of BV in women with vaginal discharge using Nugent Score and culture and evaluate agreement between these procedures.

Study Design
Descriptive and Cross-sectional study

Setting and Duration
This study was carried out at the Department of Microbiology, Fauji Foundation Hospital, Rawalpindi, Pakistan from July 2017 to December 2017.

Subjects and Methods
All females with age more than 18 years reporting the hospital outpatient department with complaint of vaginal discharge were included in the study. Sampling technique was purposive and non-probability. At least three high vaginal swabs were taken for Gram staining, culture and wet smear for *Trichomonas* vegetative form. Nugent's Score was assigned as per standard criteria. A score of ≥7 indicated BV.

Results
Age range of patients (n=203) was from 20-80 years. Mean age was 44.58 + 10.24 (Mean + SD). In 93 patients (45.81%) Nugent Score was 7-10 whereas culture of high vaginal swabs revealed positive growth in 116 patients (57.14%).Bacterial culture was better in diagnosing BV than Nugent score (p=<0.0001). No Trichomonas was seen in all the specimens examined.

Conclusion
Nugent scoring is a good, efficient and rapid method for diagnosis of BV. However, due to low positivity than that of culture negative Nugent score does not rule out the disease and needs verification with culture.

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Key Words
Bacterial vagnosis, Nugent Scoring, Vaginal discharge

Introduction

Bacterial vaginosis (BV) is a change in flora balance of vagina in which there is increase of anaerobic Gram negative rods like *Gardnerella vaginalis*and *Mycoplasmas*with a decrease in *Lactobacilli*that is primarily responsible for healthy vaginal tissue.[1] In females of reproductive age this problem often observed.[2] BV commonly presents as abnormal vaginal discharge and vulva pruritis.[2] Gram-staining devised by Nugent *et al* is acceptable technique for diagnosis of BV. Amsel's clinical criteria for BVare also quicker method in case of increased workload.[2]

Frequency of distribution is affected by various socioeconomic factors including multiple pregnancies,[3] asymptomatic pregnant women,[4] and poor hygiene,[5] previous history of spontaneous abortions and use of intrauterine contraceptive devices.[5] In most of the studies conducted in different setups most frequent microorganisms involved in BV are *Gardnerella vaginalis* and *Mycoplasma hominis*.[7,8,9] Other frequent organisms are *Escherichia coli* and *Mycoplasma curtisii*.[9]

Diagnosis of BV can be made using several methods, such as Amsel's criteria, Gram stain, vaginal cultures, oligonucleotide probes, molecular methods. The most specific and sensitive of these tests is PCR technique, but it not feasible in our country due to high cost and lack of equipment at various remote laboratories.[9] The most widely used method for diagnosing BV is Nugent scoring, especially for developing countries such as Pakistan where it has proven to be cost effective, reliable and efficient.[10]

As pathogenesis of BV is not clear, the management of BV is a challenging aspect for clinicians and throughout the world, and it is becoming a very common syndrome in women of reproductive age. Recent articles are showing variability in prevalence of BV. Hence determining frequency of distribution will help to assess the actual load of this infection. It will further help the early detection, management and prevention of

complications.

Materials and Methods

This descriptive, cross sectional study was carried out by Department of Microbiology, Fauji Foundation Hospital, Rawalpindi from July 2017 to December 2017. Females with ages more than 18 years reporting the hospital outpatient department with complaint of vaginal discharge were included in the study. Sampling technique was purposive and non-probability sampling. A sample size of 203 was taken using 95% confidence level and 5% margin of error and taking expected frequency of BV as 15.6%.[5] After taking through clinical history high vaginal swabs were collected. At least three swabs were collected which were used for slide smear and Gram staining, bacterial culture and preparation of wet smear for Trichomonas. The culture was done by inoculating the Blood agar, chocolate agar and MecConkey agar. Media were kept for 24-48 hours at 35^0C±2 and additional CO_2 was provided using candle jar for Chocolate agar. One blood agar plate was kept at 35^0C±2 an aerobically. The procedures describe by Barrow and Feltham was used for *Giardia lamblia* isolation and identification.[11] However, isolation of *Mycoplsma*, *Mobiluncus* and other fastidious microorganisms could not be adopted because of meager facilities available for them. Women with history of antimicrobial including Metronidazole intake, any oral or vaginal antifungal preparation, women in post-partum period, previously diagnosed with HIV infection, having visible vaginal or cervical mass or suspected cancer were excluded from the study. Contaminated samples were not included.

All vaginal swabs were collected with the sterile swab sticks. Vaginal speculum was used for obtaining the specimen under visual control. Swabbing was done by inserting a dry cotton-wool tipped swab in vaginal furnaces. Swabs were returned to the transport sleeve, labeled and transported to the laboratory.

Processing

The HVS specimens were considered potentially infectious and masks & gloves were always worn. In the laboratory, the swabs were smeared onto the glass slides. The smears on the glass slides were air-dried. Gram stain was performed and the stained smears were independently examined using the light microscope under oil immersion at 100x magnification lens. Nugent's Score was assigned as per criteria mentioned in Table1. A score of >7 indicated BV.

Operational Definitions

Bacterial vaginosis

If the Nugent scoring was >7, it was labeled as bacterial vaginosis (Table 1).

Nugent scoring

On Gram-staining morphology and number of micro-organism in per smear field is counted that determines the score as per table 1.

Data Analysis

The data was entered in SPSS (version 21) software. Descriptive statistics was calculated for both qualitative and quantitative variables. Mean and standard deviation (SD) was given for quantitative variables i.e; Age. Frequency and percentage was given for qualitative variables i.e; BV. Effect modifier like age, socioeconomic group, diabetes, multiple pregnancies, spontaneous abortions, intra-uterine contraceptive devices was controlled by stratification. For post stratification chi-square was applied. P-value = 0.05 was taken as significant

Results

A total of 203 patients met the inclusion criteria during the study period. Age ranged from 20-80 years. Mean age was

Table 1. Nugent Scoring Criteria for the Microscopic Diagnosis of Bacterial Vaginitis

# Lactobacillus (Gram-Positive bacilli-llarge)	Score	# Gardenerella/Baceroides (Gram variable bacilli-small)	Score	Mobiluncus species (Gram-Negative/ variable bacilli-curved)	Score
≥30	0	0	0	0	0
5-20	1	<1	1	<1	1
1-4	2	1-4	2	1-4	1
<1	3	5-20	3	5-20	2
0	4	≥30	4	≥30	2

Interpretation:Nugent Score =3 = Smear negative for Bacterial Vaginosis; **Nugent Score 4-6** = Smear indeterminate for Bacterial Vaginosis (Altered vaginal flora and repeat testing of another vaginal smear is recommended); **Nugent Score ≥7** = Smear consistent with Bacterial Vaginosis

44.6+ 10.2 (Mean + SD). Using Nugent scoring 93 patients (46%) had a score of 7-10 making the diagnosis of BV as compared to the results of cultures that tested (n=116, 57%) culture positive for BV. Bacterial culture when compared to Nugent Score for diagnosing BV had a p value of <0.0001 proving it better for diagnosis [Table 2]. The organisms isolated were (n=45, 39%) *Escherichia coli*, (n=21, 18%) *Klebsiella pneumoniae*, (n=8, 7%) *Citrobacterfreundii*, (n=5,4%) *Gardnerellavaginalis*, (n=17, 15%) *Staphylococcus aureus*,(n=11, 10%) *Streptococcus pyogenes*, (n=4, 4%) *Streptococcus* species and 5 (4.3%) *Peptococcus* species. No Trichomonas was seen on direct microscopy of all the specimens. All this makes Nugent Scoring an efficient screening tool for establishing the diagnosis with higher scores, although due to low positivity than that of culture negative Nugent score does not rule out the disease and needs further workup. Presence and absence of *Candida* species in patients with vaginal discharge did not affect the Nugent scoring results (p= 0.257) [Table 3].

Table 2: Comparison of Nugent scoring with culture results in patients with vaginal discharge (n=203)

Bacterial culture result	Nugent Scoring*			P value
	Normal # patients	Intermediate # patients	Bacterial vaginosis # patients	
No growth	23	0	8	<0.0001
Normal vaginal flora	42	4	10	
Culture Positive@	33	8	75	
Total	98	12	93	

*Nugent scoring; Normal= 0 – 3; Intermediate= 4 – 6; Bacterial vaginosis= 7 – 10;
@Culture result= Growth of pathogenic bacteria

Table 3. Association of Nugent scoring with isolation of candida species in patients with vaginal discharge (n=203)

Candida	Nugent Scoring*			
	Normal # patients	Intermediate # patients # patients	Bacterial vaginosis	Total # patients
Present	19	2	23	44
Absent	77	10	72	159
P value	0.257			
Spearman's Correlation significance	0.413			

*Nugent scoring; Normal= 0 – 3; Intermediate= 4 – 6; Bacterial vaginosis= 7 – 10; @Culture result= Growth of pathogenic bacteria

Discussion

Bacterial Vaginosis (BV) is a common vaginal disorder in women in reproductive age. Since the initial work of Leopoldo in 1953 and Gardner and Dukes in 1955, researchers have not been able to identify the causative etiologic agent of BV. However, there is increasing evidence that BV occurs when *Lactobacillus* spp., the predominant species in healthy vaginal flora, are replaced by anaerobic and microaerophilic bacteria, such as *Gardenellavaginalis*, *Mobiluncuscurtisii*, *M. mulieris*, other anaerobic bacteria and/or *Mycoplasma hominis*. It is estimated worldwide that 20–30 % of women of reproductive age attending sexually transmitted infection (STI) clinics suffer from BV, and that its prevalence can be as high as 50–60 % in high-risk populations (e.g., those who practice commercial sex work (CSW). According to epidemiological data, women are more likely to report BV if they: 1) have a higher number of lifetime sexual partners; 2) are unmarried; 3) have engaged in their first intercourse at a younger age; 4) have engaged in CSW, and 5) practice regular douching.[12]

In our study about half of the women (45.81%) reporting outpatients with complaint of vaginal discharge were found to be positive for BV using Nugent scoring. However, some other studies revealed lesser positive results using Nugent Scoring; in the study at Agha Khan University Hospital Karachi (Pakistan) frequency was 16.1%,[13] in the study at Bahawal Victoria Hospital Bahawalpur (Pakistan) was 10.8%[14] and at Khyber Medical University frequency of 35.3%[6] was noted. Similarly study conducted at Ethiopia in 2014, revealed prevalence of BV as19.4%.[1] Another study conducted in Yazd city revealed 15.6% frequency of bacterial vaginosis.[15] The variation in results of these different studies appears to be multifactorial. In some of the studies the multiple observers were involved in examination of the Nugent Scoring smear slides. In one study BV was identified if Amsel criteria and Nugent scoring both revealed positivity. The study population in our study was women having discharge whereas in other studies the women were included in the study irrespective of vaginal discharge. Moreover, in almost all these studies only females of reproductive age were included where as in our study older ages were also included.

In our study HVS cultures diagnosed more cases of BV as opposed to Nugent score with a significant p value but about half of the women with vaginal discharge revealed positive Nugent score makes it reliable and efficient way of screening especially when ruling in the disease. However, negative test

cannot rule out the disease but requires further workup.

Presence and absence of *Candida* species in patients with vaginal discharge did not affect the Nugent scoring results. A study from India conducted in 2012 in India[16] showed that the differences in the prevalence of vulvovaginal candidiasis were not observed by the presence or absence of laboratory-confirmed BV which correlates with our study (p=0.257).

Conclusion

With appropriate sampling and diagnostics, 45.81% of females with complaints of vagina discharge had BV, making it likely the most common cause of vaginal discharge in our setting. Nugent score is an efficient, cost effective and reliable method for screening of BV making the diagnosis likely if tested positive, but negative testing does not rule out the disease and needs further workup.

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ORIGINAL ARTICLE

Surveillance of multidrug-resistant organisms in a tertiary care hospital in Pakistan

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Abstract

Objective
To describe different trends of multi-drug resistant infections in a specialized consultative care hospital in Islamabad, Pakistan

Study design
Descriptive study was performed at Shifa International Hospital, during January 2015 till June 2015

Methods
Patient's history, culture reports, and medical records were reviewed during ID consultations and data analysis was done with SPSS17

Results
A total of 300 cases in six-month period were analyzed. Eighty percent were monomicrobial. The highest percentage of infections were caused by organisms like ESBL *E. Coli* (23.3%). *Methicillin resistant staphylococcus aureus* (15.7%), *Klebsiella* (13.3%), *Acinetobacter* (13%), *Enterobacter* species (10.0), *Pseudomonas* (9.7%), Vancomycin resistant enterococci (5.3%). MSSA (3.3%) Candida species (4.7 %), and *Serratia* (1.7%)

Conclusion
In this study we found a large proportion of drug resistant infections in Pakistan

Introduction
Close observation of resistant organisms in a hospital is important to optimize treatment outcome. Whenever a clinical facility is colonized with pathogens it's difficult to eradicate the species. With Increasing frequency of MDROs there is a need for continuous vigilance specially in developing countries like Pakistan. Once a bacterium is introduced in a healthcare facility its pervasiveness and spread is determined by susceptible patients, antibiotic use, and large population of affected patients ("Colonization pressure"). The patients who are immunocompromised due to underlying severe disease, surgeries or with catheters and tubes in intensive care settings are more vulnerable than patients in outpatient and day care setting. The prevention of infections will not only diminish the load of MDROs in a hospital but also decrease antimicrobial resistance and financial implications associated with these infections. There are pertinent practices that should be integrated in patient care that consists of appropriate catheter management, following sterilization techniques during procedure, meticulous diagnosis and sensible use of antibiotic will help to in control of these pathogens

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Methods
Study setting and period: The study period was from January to June 2015. Patients' hospital records were reviewed during infectious disease consultations at Shifa International, a tertiary care teaching hospital. There was a total of 300 infections, 140 patients admitted in internal medicine services, 70 in general surgery, 50 in neurosurgery, 28 in intensive care and 12 in liver transplant patients. Culture and sensitivity data from blood, pus, tracheal secretions and urine was collected from Microbiology lab.

SPSS Software version 17.0 was used to analyze the data. Mean and median age of participants was calculated. While frequencies and percentages of MRSA, Acinetobacter, *Klebsiella* spp *Enterobacter* species, Pseudomonas, *Serratia marcescens*, VRE, MSSA and Candida species were calculated in Medicine, Surgery, ICU, Neurosurgery and Liver Transplant.

Results
The highest percentage of ESBL *E. Coli* was recorded in internal medicine. 140 patients were admitted with underlying Cardiac, gastrointestinal and Renal conditions. Out of these (28%), patients had ESBL *E. Coli* in their cultures. (13%) had Methicillin Resistant *Staphylococcus aureus*. Followed by *Enterobacter* species (12%), *Klebsiella* (12%), *Acinetobacter* (12%) Pseudomonas (10%) and MSSA (8%) Lowest percentage in medicine department was of VRE (3%) Serratia (1%) and Candida (1%).

28 intensive care patients had Klebsiella (25%), *Acinetobacter* (22%), ESBL *E. Coli* (22%) Pseudomonas (12%) Methicillin *resistant staphylococcus aureus* (7%) *Enterobacter* species (3%), and VRE (7%). 50 Neurosurgery patients had *Klebsiella* (20%) ESBL E. Coli (18%) Candida (17%), *Acinetobacter* (17%), Pseudomonas (12%) Methicillin resistant *Staphylococcus aureus* (11%) *Enterobacter* species (7%) and Serratia (3%).

During the six month study period, 300 consultations were done when stratified by clinical service the most common organisms for all specialties were *ESBL E. coli*, Staphylococcus *aureus* (MRSA), *Acinetobacter* and *Klebsiella*.

Most of the infections were monomicrobial (80 %). Gram-negative organisms caused 76.3 % Gram-positive organisms caused 19% and Fungi caused 4.7 % of the cases. ESBL *E. Coli* (23.3%). Methicillin resistant *Staphylococcus aureus* (15.7%), *Enterobacter* species (10.0), *Klebsiella* (13.3%), *Acinetobacter* (13%), Pseudomonas (9.7%), MSSA (3.3%), vancomycin-resistant Enterococci (5.3%), Candida species (4.7 %) Serratia (1.7%),

Significant relationships were observed among certain specialties and infections in patients. Methicillin resistant Staphylococcus aureus was highest among general surgery patients, ESBL *E. coli* in medicine, VRE in Liver transplant, *Klebsiella* in Neurosurgery and intensive care units.

Discussion

ESBL Enterobacteriaceae is emerging problem in community as well as the patients admitted in hospitals.[1] Asia is the region in the world where ESBLS appeared de novo and frequencies of these organisms is rising every year.[2] MRSA has exceptional potential of causing outbreaks frequently in number of hospitals over many years. Different researches have shown that almost 33 Percent people have staph colonization in their nose.

MRSA is transmitted through direct contact and hence poor hand hygiene can lead to outbreaks. A simple measure of washing hands before each patient encounter can save many lethal infections caused by MRSA and save lives.[3]

Prolong stay in hospital with severe illnesses, extensive antimicrobial use and procedures are well known risk factors for Acinetobacter colonization. the mortality rate is high and now strains resistant to polymyxins are being reported, making these infections almost incurable in future.

In recent years, it's becoming common to use broad spectrum antibiotics. The antibiotic use, lack of following infection control guidelines is a risk factor for VRE infections, chemotherapy induced neutropenia, and post-transplant neutropenia is independently linked to VRE infection. In Pakistan, the first VRE was reported in 2002, about 10 vancomycin -resistant e. faecalis isolates were reported in Agha khan university hospital Karachi.[4]

Surveillance programs are important to determine resistance patterns. Rotation of antibiotics is an approach that will reduce resistance by withdrawing an antibiotic from intensive care for short period of time. These regimens will cut down this selective pressure. Awareness about emerging species and their sensitivity is crucial for management of nosocomial infections.[5]

We have a limited antimicrobial resource. The more frequent use today, the less potent they will be in future. Therefore, physicians and healthcare professionals around the world are following antibiotic stewardship. It's a promise to use the antibiotics when its needed, to select the right drug and administer with proper method. This way patients will get the therapeutic benefits, avoids un necessary harm and secure the lifesaving quality of these drugs.

Conclusion

Primarily we must prevent infections with excellent infection control practices, keep a track of emerging resistance. And we must develop new antibiotics for resistant strains.

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ORIGINAL ARTICLE

In Vitro Efficacy of Third Generation Cephalosporins against Nalidixic Acid Resistant and Multi Drug Resistant *Salmonella Typhi* and *Paratyphi* via E-strip Method.

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Abstract

Background
Samonella typhi and *paratyphi* are the strains causing typhoid which carries significant mortality in our region. There have been reports of fluoroquinolone treatment failure due to resistance. After fluoroquinolones, cephalosporins have reliable efficacy against these bacteria. We aimed to find the minimum inhibitory concentrations of cefixime and ceftriaxone against nalidixic acid resistant and multi drug resistant *Salmonella typhi* and *paratyphi* in clinical isolates of a tertiary care setting to detect the rising minimum inhibitory concentrations.

Materials and methods
This prospective, cross-sectional study was carried out in Microbiology laboratory of Army Medical College, Rawalpindi from 2010-2011. Blood cultures were dealt via standard microbiological techniques for detection of *Salmonella typhi* and *paratyphi*. Isolates were then tested against antibiotic disks of nalidixic acid, chloramphenicol, ampicillin and co-trimoxazole. Minimum inhibitory concentration of cefixime and ceftriaxone were determined by E-strip method against multidrug resistant and nalidixic acid resistant *Salmonella typhi* and *paratyphi* according to CLSI guidelines. This is an ongoing study.

Results
In the first part of this study one hundred and fifty-six isolates of nalidixic acid resistant and multidrug resistant typhoidal salmonellae were included in our study. Among these eighty-one (51.92%) were *Salmonella typhi* and seventy-five (48.08%) were *Salmonella paratyphi A.* All the isolates had minimal inhibitory concentrations of cefixime and ceftriaxone in susceptible range.

Conclusion
Both ceftriaxone and cefixime prove very effective antibiotics against nalidixic acid resistant and multidrug resistant typhoidal Salmonellae. Ceftriaxone is the therapeutic agent of choice for inpatients. While cefixime, can be given on outpatient basis due to its convenient oral administration till full emergence of cephalosporin resistant typhoidal Salmonella.

Keywords
Cephalosporins, Multidrug resistant *Salmonella*, Nalidixic acid resistant *Salmonella*.

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Introduction

The oral fecal route of transmission of *Salmonella* makes it a public health threat. There is a rising trend in prevalence of infections caused by resistant *Salmonella* in humans.[1] Asian countries account for a very high incidence of typhoid fever compared to other countries. Mortality rate for typhoid fever without appropriate antimicrobial treatment is estimated to be around 30% which reduces to as low as 0.5% with effective therapy.[2]

The earliest report of successful treatment of *Salmonella* infection dates back in 1948 when Woodward and colleagues have successfully used chloramphenicol for treatment of typhoid fever. For the next two decades, chloramphenicol remained the drug of choice. In late 1970s, the first outbreak of infections caused by antibiotic resistant *Salmonella* appeared. Co-trimoxazole, ampicillin and chloramphenicol, were the antimicrobials used in the treatment of infections caused by *Salmonella* before the 1980s.[3] Multi drug resistant (MDR) typhoidal *Salmonellae* is defined as a strain of typhoidal *Salmonella* resistant to all three first line antibiotics i.e. chloramphenicol, ampicillin and trimethoprim-sulfamethoxazole. Many outbreaks of resistant strain infections have occurred in Asian-Pacific countries, Middle East and Africa.[4] This resistance usually results from dissemination of individual MDR strains or from transfer of R-Plasmid.[5] Fluoroquinolones are usually recommended as alternatives in such cases.[6, 7] But unfortunately, some strains of typhoidal *Salmonellae* have shown reduced susceptibility to fluoroquinoloes.[6,8,9] On disc diffusion testing with the recommended break-points, organisms which are labeled

susceptible to fluoroquinolones, show poor clinical response, when treated with these agents. However, these isolates are resistant to nalidixic acid via disk diffusion method. So, in other words resistance to nalidixic acid can predict therapeutic failure of fluoroquinolones and can be used to guide antimicrobial treatment.[2] However CLSI has introduced updated breakpoints of fluoroquinolones in 2012 against typhoidal Salmonella and this has precluded the use of Nalidixic acid to screen for fluoroquinolones resistance. Azithromycin is used as an oral alternative, but resistance to this antibiotic is also emerging in different parts of the world. In this scenario, we are left with very limited options and the third generation cephalosporins prove to be a promising alternative till full emergence of cephalosporin resistant typhoidal Salmonella.[8] Keeping in view the emergence of Ceftriaxone resistant typhoidal Salmonella, the objective of our study is to determine the minimum inhibitory concentrations (MIC) of ceftriaxone and cefixime against nalidixic acid and multidrug resistant typhoidal *Salmonellae* from clinical isolates in a tertiary care setting ten years back and now compare it with current MIC of ceftriaxone and cefixime against typhoidal Salmonella.

Materials and methods

First part of this prospective cross sectional research was performed from 2010-2011 in Microbiology laboratory, Army Medical College, Rawalpindi. Proper approval was obtained from the ethical committee of the institute. One hundred and fifty-six isolates of nalidixic acid resistant (NAR) and multidrug resistant (MDR) typhoidal *Salmonella* isolated from blood of patients admitted to the Military Hospital Rawalpindi, Pakistan with a strong clinical suspicion of typhoid fever were included in our study. Total 5ml venous blood sample was drawn under aseptic precautions from adult patients and inoculated in 50ml of Brain heart infusion (BHI) broth. For children, 3 ml of venous blood was inoculated in 30ml of BHI, so that blood to broth ratio 1:10 was maintained. The cultures were incubated for 7 days at 37ºC before being reported as negative. All culture bottles were examined daily. If the bottle showed any visible sign of growth, subculture was done on blood agar and MacConkey's agar plate at day 1, 2, 5 and 7. The plates were then incubated at 37ºC for 24 hours. The typhoidal Salmonellae were identified by adopting the standard microbiological procedures which included colony morphology, gram staining results and biochemical reactions such as catalase, oxidase, urease, motility, Voges Proskauer test, citrate agar slant test, methyl red test and triple sugar iron agar test. The isolates of typhoidal Salmonellae were further confirmed by type-specific anti-sera. Further antibiotics susceptibility test of all the isolates were performed by Kirby-Bauer disc diffusion technique.[10] The Mueller Hinton agar plates with antibiotic discs were incubated at 37ºC and zone of inhibition around the antibiotics were measured after 18 hours and 24 hours of incubation. The routinely used antibiotics were amoxicillin, azithromycin, aztreonam, cefixime, ceftriaxone, ciprofloxacin, co-trimoxazole, nalidixic acid. All NAR and MDR typhoidal salmonellae were tested for the determination of minimum inhibitory concentrations (MICs) of cefixime and ceftriaxone by using E strips. A well isolated colony from an overnight agar plate was emulsified in a suitable suspension medium to achieve inoculum turbidity comparing to 0.5 McFarland turbidity standards. Inoculum was applied with a sterile swab on sensitivity agar. Swab was streaked on agar plate thrice to evenly distribute the inoculum. After the agar had dried, E-test strips were applied to the inoculated agar surface with a pair of sterile forceps. After the required incubation period and only when an even lawn of growth was distinctly visible, the MIC values were read and were interpreted according to the criteria set by CLSI.[10] MIC 90 and MIC 50 were calculated.

Results

One Hundred and fifty-six NAR and MDR typhoidal salmonellae were included in our study. Among these eighty-one (51.92%) were *Salmonella typhi* and seventy-five (48.08%) were *Salmonella paratyphi A.* Minimum inhibitory concentrations of both ceftriaxone and cefixime were insusceptible range. MIC results of ceftraixone and cefixime are shown in table 1. Table 2 and Fig 1 show the range, MIC 50 and MIC 90 of ceftriaxone and cefixime against MDR and NAR typhoidal Salmonellae.

Unpaired t test was applied on MIC of Ceftriaxone and Cefixime against Salmonella Typhi and Paratyphi and no statistically

Table 1. Minimum inhibitory concentrations of cefixime and ceftriaxone against MDR and NAR typhoidal Salmonellae (n=100)

Antimicrobial agent	% of isolates susceptible at MIC (µg/ml)										
	0.016	0.023	0.032	0.047	0.064	0.094	0.125	0.19	0.25	0.38	0.50
Ceftriaxone	0	3	3	20	52	10	9	3	0	0	0
Cefixime	0	2	2	4	18	51	14	7	2	0	0

Table 2. Range, MIC 50 and MIC 90 of cefixime and ceftriaxone against MDR and NAR typhoidal *Salmonellae*.

Antimicrobial agent	Range	MIC 50	MIC 90
Ceftriaxone	0.023-0.19	0.064	0.125
Cefixime	0.023-0.25	0.094	0.125

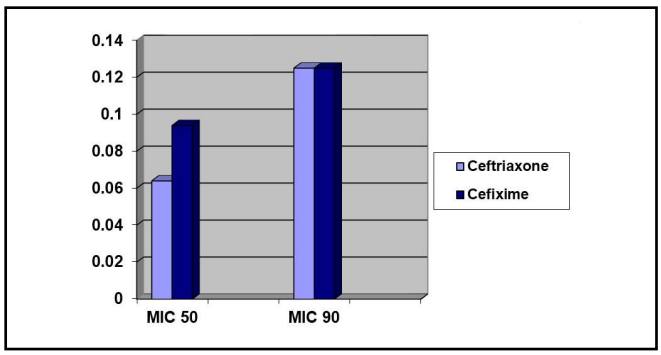

Fig 1. Graph showing comparison of MIC 50 and MIC 90 of cefixime and ceftriaxone.

significant difference between the MIC of two groups was found and thus Ceftriaxone and Cefixime may either be used in these isolates till full emergence of ceftriaxone resistance.

Discussion

Our results show that ceftriaxone and cefixime have same in vitro efficacy against NAR and MDR typhoidal salmonellae. These microbiological results supplement the available data, which showed cefixime as an efficacious and suitable oral alternative, even in cases of MDR *S. typhi*. Our results are similar to a study carried out in Bangladesh in year 2008 which also showed 100% sensitivity of typhoidal *Salmonellae* against ceftriaxone and cefixime.[11] A study done in Egypt also concluded that ceftriaxone was most effective for admitted patients, because of rapid clinical cure, and cefixime was the most effective for oral therapy for outpatients.[12] All the typhoidal *Salmonella* strains were uniformly susceptible to ceftriaxone, cefpodoxime and cefixime in a study carried out in India in 2008.[13] A study done in Bangladesh, Indonesia, Taiwan and Vietman has revealed that not a single isolate was resistant to ceftriaxone.[14] In medical literature, a few case reports have been noted reporting isolates of typhoidal *Salmonella* resistant to third generation cephalosporins. Such cases are reported sporadically including Pakistan.[15, 16, 17] However, recent studies on large sample sizes conducted in big cities of Pakistan, have shown that the resistance to these agents is still low.[18-21] Resistance to ceftriaxone varies from 0% to 3.3% and for cefixime ranges between 0% to 13.3%.

Clinical studies have shown excellent in vivo efficacy of cefixime and ceftriaxone against enteric fever.[22] Cefixime is the first extended spectrum cephalosporin which is available as oral formulation. This antibiotic has a strong activity against *Salmonella* strains causing typhoid (MIC^{90} of 0.25 µg/ml), and its clinical usefulness has also been proven in many studies. This activity is comparable to sister drug ceftriaxone as well.[23]

If the cost of oral cefixime is to be compared with ciprofloxacin then there is difference of only around 187 PKR or 1.7 USD, so we still have an alternative still affordable and with the same ease of oral administration till full emergence of ceftriaxone resistant typhoidal Salmonella. To conclude, both ceftriaxone and cefixime prove very effective antibiotics NAR and MDR typhoidal Salmonellae. Ceftriaxone is the therapeutic agent of choice for inpatients. While cefixime, can be given on outpatient basis, due to its convenient administration.

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Acknowledgements

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Limitations of study

Limitations are the isolates are from year 2010-2011 but this data will help us compare the recent rising trend of ceftraixone and cefixime resistance in Typhoidal Salmonella in our region.

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REVIEW ARTICLE

New and Emerging Challenges in Management and Control of Typhoid Fever

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Key words
typhoid, enteric fever, XDR typhoid, emerging drug resistance

Introduction

Enteric fever, generally known as typhoid, is an extremely common infection in developing countries where environmental hygiene is vastly compromised, and where children are most vulnerable to the infection.[1] Enteric fever is caused mostly by typhoidal *Salmonella*, including *Salmonella enterica* serotype *typhi*, and less frequently by serotypes *paratyphi* A, B, and C. It most frequently manifests as nonspecific, prolonged fever without localization. Clinically the differential diagnosis for acute fever is extensive and includes a number of bacterial, viral, fungal, and parasitic infections, as well as noninfectious entities. An experienced physician should be able to rule out other causes of acute febrile illness on the basis of clinical history, examination and targeted laboratory tests before considering typhoid as the cause of acute fever without localization.

Microbiological characteristics

Salmonella enterica are gram negative, encapsulated, motile bacilli with flagellae, and belong to the family Enterobacteriacae.[2] Preliminary blood, bone marrow, urine or stool cultures on MacConkey agar yields white or colorless colonies that do not ferment lactose (NLF). There are no known animal reservoirs of typhoidal *Salmonella*, and the source of infection is organisms shed in the stool of infected humans that are acquired from contaminated food or water, or poor hygiene after using the toilet.[3] The most common mode of transmission remains municipal water contaminated with sewage. Chronic asymptomatic bacterial shedding in the stool with gallbladder disease may contribute as risk factor for carriage, although this risk has not been substantiated in poorer countries.[4]

Salmonellae have been grouped into six serogroups and more than 2500 serotypes, according to diverse surface structures. This system of classification has allowed for differentiation between *Salmonella enterica* and non-typhoidal salmonellae (NTS). NTS are present in other animal species as well as in the environment and cause episodic food- borne diarrheal outbreaks.[3,5]

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Gastric acid is protective against salmonella, and reduction in gastric pH due to prolonged use of H_2 inhibitors and proton pump inhibitors (PPI) are likely to favor salmonella infection. There is also an association of *Helicobacter pylori* infection with salmonella, both probably related to unhygienic food and water.[6]

Symptoms

7-14 days after ingestion of contaminated food or water, the bacteria invade and multiply in Peyer's patches in the ileum, during which the patient is either asymptomatic or has vague abdominal pain, diarrhea and/or constipation, and a feeling of being 'unwell'. Fever rises gradually over several days, rising up to 40^0 C with chills. There is a dull constant headache with generalized myalgia, nausea, occasional vomiting and dull, poorly localized abdominal pain. If infection persists untreated, the liver and spleen become palpably enlarged.[5] A useful clinical tip-off in examination in the adult is measurement of pulse and temperature simultaneously to appreciate relative bradycardia (normally, for each degree rise of body temperature above 100^0 F the pulse rate rises by 10 beats per minute); however, this feature is not consistent, as complications such as abscess or overt or occult bleeding may mask this clinical finding. Moreover, temperature: pulse dissociation, is not a feature of typhoid in children.[7,8]

If the infection remains untreated in the second to fourth week, the patient gets increasingly sick with higher fever, weight loss, progressive weakness, and complications may set in as the bacteria metastasize to various viscera. Gastrointestinal bleeding due to erosion of necrotic ileal Peyer's patches through the wall of enteric vessels may lead to a fall in hematocrit and reactive leukocytosis. Blood transfusions may be required, especially if a large blood vessel is eroded. Intestinal perforation, usually of the ileum, and not infrequently of the colon, may be an ominous complication requiring urgent surgical intervention.[9] The clinician should be on guard if the patient appears restless with further rise of temperature, along with tachycardia, hypotension, rise in white blood count, and the chest X-ray may reveal free gas under the diaphragm, indicating intestinal perforation. Feculent fluid may fill the abdomen.

There may be mental confusion, somnolence, or agitation and delirium, progressing to obtundation. Other complications include cholecystitis, hepatitis, pneumonia, acute kidney injury, and myocarditis. Children may develop typhoid meningitis or osteomyelitis.[10] Unrecognized and untreated typhoid carries a mortality of 10-30%, but with timely treatment the fatality rate

is less than 1-4%.[11]

Diagnosis

It is important to confirm the diagnosis through isolation of the organism on culture, which also provides sensitivity testing to antibiotics. Blood culture, if drawn before antibiotic administration and in sufficient quantity, gives a yield of 80% in the first 1 to 2 weeks.[12] The yield of positivity falls to below 40% if antibiotics are taken before phlebotomy is performed.[13] Bone marrow culture is likely to yield positivity even if the disease is prolonged, severe or complicated, because of higher bacterial concentration in the marrow. Culture yield from blood and bone marrow is considerably improved with use of Brain Heart Infusion broth medium.[13] Understandably, bone marrow aspiration is not readily acceptable to patients, but may be offered when the cause of prolonged fever remains elusive. *Salmonella enterica* can be isolated from stool in 30% cases, and in urine a low 1%.[12,13,14,15,16,17] In culture negative cases, PCR, if available, may be a useful tool for diagnosis.[18]

Several serologic tests have been in vogue for diagnosis of typhoid fever, the most widely used being the Widal test, which measures agglutinating antibodies against O and H antigens of S. *typhi* and paratyphi. Due to lack of standardization and its low specificity, the Widal test is frequently misinterpreted, and this test is now considered obsolete.[14,19]

Another popular serologic test, Typhidot®, which measures IgM and IgG antibodies against 50 kDa outer membrane protein of S. *typhi*, is purported to be a rapid diagnostic test with reported sensitivity of 67-98%, and specificity 85-90%. However, because of cross reactivity with other gram-negative bacteria as well as background antibody levels in the general population, the Typhidot® may remain positive indefinitely, even in uninfected people. A recent systematic review of studies of two widely used tests, Tubex TF and Typhidot[18, 20] concluded that the performance characteristics did not justify their use. Widespread prescription of these tests has, unfortunately, led to enormous misuse of antibiotics, rendering precious antibiotics redundant, incurring huge expenses to the patient and causing financial anxiety to less affluent patients. For suspected typhoid a single blood culture before starting antibiotics is the gold standard and the best and most cost-effective option. The accompanying sensitivity report is essential for guiding the physician toward antibiotic selection. This becomes all the more important in today's conditions of emerging resistant bacterial infections.

Routine laboratory tests are not specific but must be done to rule out sepsis, malaria, and prevalent viral infections. The white blood count is usually in the low to normal range, and ALT/AST may be 3-5 times upper limit of normal. Leukocytosis in typhoid is suggestive of a complication such as intestinal perforation, and a fall in hemoglobin is indicative of intestinal bleeding.[3,5]

Antimicrobial Selection

Prior to 1970s the drugs of choice were ampicillin, cotrimoxazole and chloramphenicol, and these were used effectively for cure for several decades.[21,22] Plasmid mediated resistance to chloramphenicol was first reported from Mexico, and subsequently from Vietnam, India, Bangladesh and South Korea. Ampicillin and cotrimoxazole were used increasingly until 1980s when multi drug resistance (MDR) to all three drugs became worldwide. A study from India in 2005 showed MDR typhoid as high as 66%.[23] Bhutta *et al*. reported increased mortality among children with MDR Salmonella infection.[1] As a response to increasing cases of typhoid in developing countries, fluoroquinolones were introduced, and ciprofloxacin became the drug of choice. In 1992 the first case of fluoroquinolone resistant typhoid was reported from UK, followed by reports from other countries. MIC ≥ 128 µg/ml was reported from Japan in 2002.[24] Nalidixic acid was used as surrogate for fluoroquinolone susceptibility; however, by 2011 15-36% resistance to fluoroquinolones was reported from several parts of the world.

Following resistance to fluoroquinolones, extended-spectrum cephalosporins, such as ceftriaxone and cefuroxime were used successfully in typhoid; however, reports of their resistance against *salmonellae* started appearing from South East Asia as early as in mid 1980s.25,26 Of growing concern was the fact that several regions were reporting carbapenem resistant NTS. In 2018 Klemm *et al*[27] identified 80 XDR S. *typhi* isolates by whole-genome sequencing and found large numbers of resistance determinants.

Several studies from various countries have reported *in vitro* azithromycin sensitivities against *Salmonella enteric*a, with MICs among human isolates ranging from 1 to 32 µg/ml.[28,29] Randomized clinical trials (RCTs) have established the azalide antimicrobial azithromycin to be an effective alternative oral treatment for uncomplicated enteric fever.[29, 30, 31,32] It belongs to the macrolide group of antibiotics, has excellent penetration into most tissues, and achieves very high concentration in polymorphonuclear leukocytes and macrophages. Hence it is considered to be an excellent choice of treatment against *Salmonella enterica*.

However, a case report from UK in 2010 reported failure of azithromycin against *S. paratyphi* with MIC 1: 256 µg/ml. The patient had acquired the infection in Pakistan.[33] There are no clinically validated MIC breakpoints for azithromycin against salmonellae, therefore no established guidelines exist for treating typhoid with azithromycin.[29]

The most common mechanisms of macrolide resistance are efflux of the antibiotics (extrusion of the drug from the cell) and target site modification by a post-transcriptional modification of 23S rRNA, or mutations in 23S rRNA of ribosomal proteins. Studies investigating azithromycin resistance mechanisms in

Salmonella are scarce, and it is recommended to conduct further studies. The detection of resistance mechanisms is becoming increasingly important as surveillance programs recognize their role in the global control of antimicrobial resistance.[27,28]

The transitioning from drug susceptible to drug resistant salmonella almost every decade raises the specter of further drug resistant S. *typhi* and we may face increasingly untreatable infections. The first case of ceftriaxone resistant S. *typhi* was reported in November 2016 in Hyderabad, Pakistan.[27] The isolate was also resistant to ampicillin, cotrimoxazole, chloramphenicol, fluoroquinolones, and third-generation cephalosporins, leaving few options for treatment. The resistance to five classes of antibiotics is henceforth referred to as extensively drug resistant (XDR). Genetic classification of the isolate was done through whole genome sequencing, and the results revealed the bacterium to be of indigenous origin rather than of imported source. It was suggested that an MDR H58 clone acquired an ESBL plasmid from an E. *coli* or an enteric bacterial donor. The emergence of the MDR H58 clone is a watershed event in the evolution of drug resistant S. *typhi* and leads to a worrisome outlook for a common but potentially dangerous infection that affects vast swathes of both pediatric and adult populations in Pakistan. Clusters of XDR typhoid have emerged, as cases from both urban and rural populations are escalating, while physicians mainly from Sindh province continue to report cases of XDR S. *typhi,* especially among children from the lower socioeconomic class. Over one year from July 2017 to August 2018, 1221 culture proven S. *typhi* were diagnosed in the lab at The Indus Hospital in Korangi, Karachi, of which 627 (51%) were XDR. Children below age 15 years overwhelmingly accounted for these cases (oral communication, publication in progress.) Many children and adults have presented with jaundice, anemia, soft tissue abscess, intestinal bleed or perforation. The more toxic patients are managed with intravenous meropenem with or without azithromycin and at least a few have required surgical or radiological intervention.

For patients with invasive XDR *Salmonella* infection, carbapenems may represent the last resort. An article reports carbapenem resistant *Salmonella enterica* due to plasmid mediated Class A cabapenemase KPC-2.[34] The emergence of carbapenem resistance in *Salmonella*, will pose a serious clinical problem, owing to the lack of other therapeutic choices.

The rampant irrational use of antibiotics in Pakistan has contributed hugely to multidrug resistant bacteria- be it gram positive cocci, gram negative bacilli, fungi or Mycobacterium tuberculosis. Not only are second line drugs not as efficient as first line drugs in most instances, they are much costlier, and many have serious adverse events. Watching the progression of antimicrobial resistance, there is now a sense of foreboding among physicians about the future management of infectious diseases, especially in hospital settings. Total drug resistant S. *typhi* will become inevitable if we continue to misuse antibiotics. The use of azithromycin or other macrolides for viral respiratory infections must be strongly discouraged.

Typhoid vaccine

Three types of typhoid vaccines are licensed by WHO:

i) oral, live attenuated Ty21a vaccine: to be taken as 3 doses over a week for children above 6 years age.
ii) injection of unconjugated Vi polysaccharide (ViPS) vaccine every 3-7 years;
iii) typhoid conjugate vaccine (TCV);

i and ii need to be repeated every 3 years.[35] TCV (Typbar-TCV®), a Vi-tetanus toxoid conjugate vaccine manufactured by Indian company Bharat Biotech, is the first typhoid conjugate vaccine to achieve WHO prequalification. The vaccine, currently licensed in India and Nepal is recommended as a single, intramuscular dose, and elicits a robust immune response in infants as young as six months of age. Typbar-TCV® offers advantages over currently available typhoid vaccines, including the ability to provide longer-lasting protection, requires fewer doses, and can be administered to adults and children younger than two years of age, making it the first-ever typhoid vaccine to be approved for this age group.[35] WHO recommends its introduction for infants and children over six months of age in typhoid-endemic countries and it can be delivered through routine childhood immunization programs and affords better protection for younger children.[36] There is no effective vaccine against paratyphoid A currently available commercially. At the time of this writing, Typbar-TCV® is not available in Pakistan, but is being applied for registration and importing. Vaccinating the entire vast population against typhoid may not be a practical option but must be done at individual or community level as far as possible.

Summary

Typhoid fever is a common enteric infection in Pakistan, affecting all ages, but more so among children. It presents as high fever without localization, and untreated or poorly treated, may result in serious complications. Diagnosis must be sought within the first week or two through blood culture *before* antibiotic is administered. Because of rising drug resistance, isolation of the organism along with drug sensitivity testing is crucial for selecting correct therapy. Laboratories in good standing no longer perform serological tests such as Widal or Typhidot®, and the Medical Microbiology and Infectious Disease Society of Pakistan (MMIDSP) strongly urges its discontinuation from all laboratories. The greatest challenge to typhoid control lies with city municipalities, whose responsibility is to provide clean drinking water. Secondly, random and imprudent use of antibiotics among humans must be discouraged, and restriction

of antibiotics for animal use must be regulated in order to retain drug sensitivity against pathogens. Stringent individual infection control practices, along with extensive reach of conjugate typhoid vaccination will prevent the infection. Control of typhoid outbreak must be dealt with urgently.

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CASE REPORT

Successful Treatment of Hepatitis C in Renal Transplant Recipient. A Case Report from Pakistan

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Abstract

The treatment of hepatitis C is a therapeutic challenge in renal transplant recipients. Interferon based regimens are relatively contraindicated due to their side effects and high risk of graft failure. With the availability of direct antivirals the situation has changed now and sustained virological response (SVR)>90% had been achieved in patients with normal kidney function. There is limited data available on the safety of these agents in renal transplant recipients. Here we report a case of a kidney transplant recipient with Hepatitis C genotype 3 infections successfully treated with Sofosbuvir and Daclatasvir based regimen. This is the first reported case from Pakistan.

Key-words

hepatitis C, Sofosbuvir , renal transplant

Introduction

Chronic hepatitis C is the one of the commonest causes of chronic liver disease around the globe with approximately 170 million people affected worldwide[1] and in Pakistan the estimated sero-prevalence of hepatitis C ranged from 2.3 % to 28.6%.[2] It is also observed that the prevalence of hepatitis C in patients with end stage renal disease (ESRD) exceeds that in the general population and the reason being the greater exposure to blood products and breach in the standard precaution in haemodialysis units.[3,4] Multiple studies have shown that HCV infection is associated with increased mortality and adverse clinical outcomes in patient with ESRD[5] and renal transplant in patients with HCV infected ESRD patients is associated with improved survival.[6] Before the availability of direct acting antivirals, the treatment of hepatitis C was hampered by the toxicity of interferon and ribavirin in these patients and it was considered a therapeutic challenge. Now with the significant progress made in the development of direct acting antivirals, the clinical scenario has changed and many patients with ESRD and kidney transplant recipient are now candidates for the treatment. There is a paucity of data regarding safety of Sofosbuvir based regimens in post renal transplant patients but a few centers have tried them with excellent outcomes. Here we report a case of Hepatitis C genotype 3 infected renal transplant recipients successfully treated with Sofosbuvir and Daclastivir for 12 weeks.

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Case History

A 56 year old male, known to have diabetes and hypertension, presented to us in clinic as a candidate for renal transplantation in December 2016. He was suffering from end stage renal disease, and was on maintenance hemodialysis, twice weekly for the prior 2 years. He was also taking fixed dose insulin for his diabetes, and was on Amlodipine 10 mg /day for hypertension. On further evaluation it was found that he was hepatitis C reactive and he had received treatment for hepatitis C in November 2015 with pegylated interferon and Sofosbuvir 400 mg once a day for 6 months and achieved end of treatment response. The HCV RNA quantitative and qualitative were ordered and it was found that he had hepatitis C genotype 3 infection and his viral load was 367,000 copies /ml . He had no signs of liver decompensation and his liver function tests showed bilirubin (total) 0.68, bilirubin (direct) 0.45, bilirubin indirect (indirect) 0.23, Gamma GT 450, SGPT(ALT) 30 U/L and alkaline phosphatase 387. The patient's Metavir score (for fibrosis) was F3. He was Hepatitis B surface antigen negative, HIV nonreactive and CMV Ig G positive. The patient had a renal transplant in March 2017 and received a kidney from his son. His son was seropositive for CMV. After 4 weeks post-transplant the patient was started on direct acting antivirals i.e. Sofosbuvir 400 mg once and a day and Daclatasvir 60 mg once day. The patient was also given cyclosporine 150 mg twice day and Mycophenolate mofetil 720 mg twice a day as immunosuppressant. Two month after transplant the patient's CMV PCR was checked and it showed rising trends and he was started on oral Valganciclovir 900 mg once a day. The patient continued Valganciclovir with Sofosbuvir and Daclatasvir and no adverse effects were noted during course of treatment except mild mucositis which was unrelated to hepatitis C therapy. His CMV PCR after 2 weeks of therapy with Valganciclovir was negative. His HCV PCR after 12 weeks of therapy came out negative. At 6 months follow up the repeat HCV PCR was negative and sustained viral response was achieved.

Discussion

Treating chronic hepatitis C in renal transplant recipients was challenging before the availability of direct acting antivirals. The treatment with Pegylated interferon and Ribavirin was difficult in kidney transplant recipients due to toxicity of the regimen and risk of graft rejection in this population.[7] We observed that the direct acting antivirals Sofosbuvir and Daclatasvir were tolerated by our patient very well with no adverse effects. We decided to treat him post renal transplantation because of the availability of data on the safety of these regimens

in both renal and liver transplant recipients and achieving sustained virological response comparable to that in general population. Sofosbuvir is eliminated by kidneys however no dose adjustment is required if creatinine clearance is >30ml/min that is why we opted to start this treatment post-transplant when his creatinine clearance improved and patient's kidney function was normalized. Kamar N et al, in his case series of 25 hepatitis C reactive renal transplant recipients described the success of Sofosbuvir based regimen. All the patients in this case series were viral load negative at the end of 12 weeks therapy and all had sustained virological response as well.[8] Lin MV et al in their retrospective analysis of 24 kidney transplant recipients who received Sofosbuvir based direct acting antivirals for hepatitis C mentioned SVR rates of 91%.[9] Sofosbuvir and Daclatasvir are considered safe with immunosuppressants and appear not to alter the pharmacokinetic profile of tacrolimus and cyclosporine.[10] In our patient, no interaction was seen between immunosuppressants and antiviral therapy and no dose adjustment was required throughout the therapy because of drug drug interaction. We reported this case to share our success story of achieving virologoical response rapidly in patient who had many co morbidities and was on immunosuppressant as well. We started therapy in the early post-transplant period when patient is on high dose of immunosuppressants, large clinical studies are needed to determine the appropriate time to initiate the therapy and the effect of early therapy on the graft outcomes.

Conclusion

Direct acting antivirals are effective and safe in treating hepatitis C post renal transplantation

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Instructions to Authors

Scope

The Infectious Diseases Society of Pakistan sponsors the Infectious Disease Journal of Pakistan (IDJ). The Journal accepts Original Articles, Review Articles, Brief Reports, Case Reports, Short Communications, Letter to the Editor and Notes and News in the fields of microbiology, infectious diseases, public health; with laboratory, clinical, or epidemiological aspects.

Criteria for publication

All articles are peer reviewed by the IDSP panel of reviewers. After that the article is submitted to the Editorial Board. Authors may submit names and contact information of 2 persons who potentially could serve as unbiased and expert reviewers for their manuscript, but IDSP reserves the right of final selection.

Submission of the Manuscript

Manuscripts must be formatted according to submission guidelines given below, which are in accordance with the "Uniform Requirements for Manuscripts Submitted to Biomedical Journals" (originally published in *N Engl J Med* 1997;336:309-15). The complete document appears at www.icmje.org. Please submit one complete copy of the manuscript and all enclosures to **The Managing Editors, Infectious Diseases Journal of Pakistan, Department of Pediatrics & Child Health, The Aga Khan University, Stadium Road, P.O. Box 3500, Karachi 74800, Pakistan.** An electronic copy of the manuscript must also be sent to pak_idj@yahoo.com**.** All manuscripts submitted to IDJP must be accompanied by an Authorship Declaration stating that *'The authors confirm that the manuscript, the title of which is given, is original and has not been submitted elsewhere. Each author acknowledges that he/she has contributed in a substantial way to the work described in the manuscript and its preparation'*. Upon submission a manuscript number will be assigned which should be used for all correspondence.

Manuscript Categories

I. Original Articles

Articles should report original work in the fields of microbiology, infectious disease or public health.The word limit for original articles is 2000.

Title page

This should list the (i) title of the article, (ii) the full names of each author with highest academic degree(s), institutional addresses and email addresses of all authors. (iii) The corresponding author should also be indicated with his/her name, address, telephone, fax number and e-mail address. (iv) A short running title of not more than 40 characters (count letters and spaces) placed at the foot end of the title page.(v) a conflict of interest statement should also be included in this section.

Abstract

Abstract should not exceed 250 words and must be structured in to separate sections headed *Background, Methods, Results and Conclusions*.

Please do not use abbreviations or cite references in the abstract. A short list of four to five key words should be provided to facilitate.

Background

The section must clearly state the background to the research and its aims. Controversies in the field should be mentioned. The key aspects of the literature should be reviewed focusing on why the study was necessary and what additional contribution will it make to the already existing knowledge in that field of study. The section should end with a very brief statement of the aims of the article.

Materials and Methods

Please provide details of subject selection (patients or experimental animals). Details must be sufficient to allow other workers to reproduce the results. The design of study and details of interventions used must be clearly described. Identify precisely all drugs and chemicals used, including generic name(s) and route(s) of administration All research carried out on humans must be in compliance with the *Helsinki Declaration*, and animal studies must follow internationally recognized guidelines. The authors are expected to include a statement to this effect in the Methods section of the manuscript. A description of the sample size calculation and statistical analysis used should be provided.

Results

Present results in logical sequences in the text, tables and illustrations. Articles can have a maximum of 5 illustrations (in a combination of figures and tables) per article. The results should be in past tense and repetition of results presented in the tables should be avoided. Exact *P*-values should be reported along with reporting of OR and RR with their Confidence Intervals where applicable.

Discussion

Emphasize the new and important aspects of the study and conclusions that follow from them. Do not repeat the details from the results section. Discuss the implications of the findings and the strengths and limitations of the study. Link the conclusions with the goals of the study but avoid unqualified statements and conclusion not completely supported by your data.

Acknowledgments

Acknowledge any sources of support, in the form of grants, equipment or technical assistance. The source of funding (if any) for the study should be stated in this section. Please see below for format of **References, Figures and Tables**.

II. Review Articles
Authoritative and state of the art review articles on topical issues are also published, with a word limit of 2000. It should consist of critical overview of existing literature along with reference to new developments in that field. These should be comprehensive and fully referenced. Articles should contain an Abstract; Main Text divided into sections, Conclusions and References.

III. Brief Reports
Short clinical and laboratory observations are included as Brief Reports. The text should contain no more than 1000 words, two illustrations or tables and up to 10 references.

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Instructive cases with a message are published as case reports. Routine syndromes or rare entities without unusual or new features are invariably rejected. The text should contain no more than 1000 words, two illustrations or tables and up to 10 references. The authorship should not exceed 3-4 persons.

V. Letter to the Editor
These may relate to material published in the IDJP, topic of interest pertaining to infectious diseases, and/or unusual clinical observations. A letter should not be more than 300 words, one figure and 3-5 references.

VI. News and Views
Informative, breaking news updates in infectious diseases from around the world (approx. 200 words).

VII. Notices
Announcements of conferences, symposia or meetings may be sent for publication at least 12 weeks in advance of the meeting date. Details of programs should not be included.

References
Number references consecutively in the order in which they are first mentioned in the text. Identify references in text, tables and legends by Arabic numerals (in superscript). References cited only in tables or in legends to figures should be numbered in accordance with a sequence established by the first identification of the particular table or illustration. Bibliography should be given in order. Authors, complete title, journal name (Abbr), year, vol, issue, page numbers. According to "Uniform Requirements of Manuscripts submitted to Biomedical Journals", as cited in N Engl J Med 1997; 336:309-15.

Tables and Figures
Data reported either in a table or in a figure should be illustrative of information reported in the text, but should not be redundant with the text. Each table must be presented on a separate sheet of paper and numbered in order of appearance in the text. Table should be numbered consecutively in Arabic numerals. Tables and Figures legends should be self-explanatory with adequate headings and footnotes.Results which can be described as short statements within the text should not be presented as figures or tables.

Illustrations
Illustrations should be numbered, given suitable legends and marked lightly on the back with the author's name and the top edge indicated. Original drawings may be submitted although high quality glossy photographs are preferable. They should be kept separate from the text. If possible, figures should be submitted in electronic format as either a TIFF (tagged image file format) or JPEG format. Minimum resolution for scanned artwork is:

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Plagiarism
Authors should refrain from plagiarism and should double check their work before submitting it for publication. Adequate references should be provided for text from other sources.

Authorship criteria
Those who have contributed sufficiently to the conceptualization, design, collection and analysis of data and writing of the manuscript should be granted authorship. Ideally all authors should be from the same department except for studies that are multi center or multispecialty.

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Department & Division

Field of Interest

Designation | PMDC No.

Phone No. Resdence | Office

Cell | E-mail

Degree/ Diploma:

☐ MBBS ☐ MD ☐ MSc Biological Scinec ☐ BSc Nursing
☐ MRCP ☐ MCPS ☐ FRCS ☐ FCPS
☐ MRCPath ☐ Ph. D ☐ M. Phil ☐ Pharma. D
☐ DCH ☐ Diplomat American Board of
Other

Application for member as

☐ Full Member Rs.3000/- for life
☐ Overseas Member US$.100/- for Life
☐ Associate Member Rs.3000/- for life

Signature Date

For Office Use Only

Approved/ Not Approved

Membership No: _____ Reference No: _____

Comments: _____

Signature General Secretary: _____

FULL MEMBERSHIP:

Should be at least medical graduates registered with PMDC and having postgraduate qualification in any field.

ASSOCIATE MEMBERSHIP:

Ph. D, Master degree & M. Phill in biological sciences, BSc in Nursing & allied medical science.

PRIVILEGES OF MEMBERSHIP:

FULL MEMBER:

All the members shall have the right to:
1. Participate in all activities of the society.
2. Vote according to constitution of the society.

ASSOCIATE MEMBERS:

All the members shall have the right to:
1. Participate in programs of the society.

Please send your Application form by hand or by mail only.
Membership fee will only be received in cash/ on-line deposit / pay order or bank draft made out to Infectious Disease Society of Pakistan.

Mailing Address and Contact Nos:

Medical Microbiology & Infectious Diseases Society of Pakistan
21 G /1, Block - 6, P.E.C.H.S., Shahrah-e-Faisal, Karachi. E-mail: mmidsp123@yahoo.com