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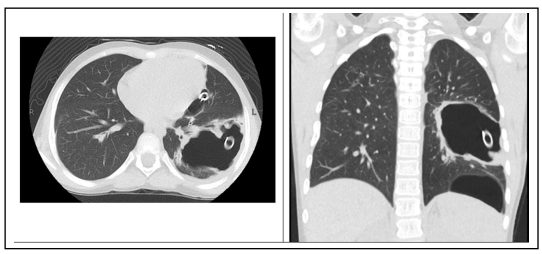

10 year/M, known case of Chronic Granulomatous disease admitted with fever, shortness of breath and increasing breathing difficulty over 3-4 weeks. Chest x ray showed pneumothorax, CT Chest confirmed the pneumothorax, collapse and consolidation with effusion. The child underwent video assisted thoracoscopy and decortication. The culture grew *Aspergillus flavus* and MRSA. This is post-surgical CT chest with contrast showed collapsed lung, pneumothorax, and bullae with two chest tube placement (A). Child is currently on Voriconazole and linezolid.

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

Safe water –Basic Human Right

Water-related diseases encompass both communicable and non-communicable diseases. The most vulnerable populations to these diseases world-wide are children, women and those living in poverty. WHO estimates that access to safe water would be able to prevent 1.4 million childhood deaths due to diarrhea, 0.5 million from malaria, 0.86 million child deaths from malnutrition and 0.28 million from drowning; another 10 million people could be saved from serious disability due to lymphatic filariasis and trachoma. Improving water, sanitation and hygiene has potential to reduce global disease burden by 9.1% and deaths by 6.3%.[1] The 64th World Health Assembly, 2011, discussed water, sanitation and hygiene as one of their agenda items[2], urging member states to recognize it as a major preventive measure towards achieving Millennium Development Goals 7 (Ensure environmental sustainability), Goals 4 (Reduce child mortality), 5 (Improve maternal health) and 6 (Combat HIV/AIDS, malaria and other diseases). However, in Pakistan, the drive to ensure access to clean water appears to be non-existent.

In Pakistan, the major water-related diseases which are wreaking havoc with our public health are enteric fever, polio, diarrhea, mosquito-borne diseases including malaria, dengue and chikungunya, and primary amoebic meningoencephalitis with *Naegleriafowleri*. The Weekly Field Epidemiology Report for July 23-29, 2018 listed 738 dengue and 84 chikungunya cases from Pakistan this year, when the monsoon season has not even started.[3] Yet another case of PAM has been reported in a 5-year-old, bringing the total number of Naegleria cases to 4 in 2018. Typhoid cases this year were 3,018, with 1,406 XDR-typhoid, resistant to first-line, quinolone and third generation cephalosporins. The most affected age group was 3-4 year-olds, with an attack rate of 22 cases / 100,000, primarily from Karachi. Another outbreak of acute watery diarrhea in Quetta affected 333 individuals with highest attack rate of 35/1000 among 1-4 year-olds. Confirmed polio cases reported in 2014 were 306, though they went down to 8 cases in 2017[4], however acute flaccid paralysis cases were 113 this year, majority being GuillianBarre syndrome[5], which may be a sequelae of gastroenteritis with campylobacter. These data suggest that water and sanitation related infectious diseases are a serious burden in Pakistan.

Major reasons for unsafe water in Pakistan are lack of infrastructure and planning, poor utilization of existing resources, water mafia in urban centers, and population growth. Guidelines are available for developing drinking-water quality regulations and standards which also provide guidance on how to monitor compliance to them.[6] Political will is needed to drive this agenda at a national level and thus ensure its implementation. Separating waste water lines and drinking water supply, regular repairs and most importantly treating waste water before releasing into the water sources.

Awareness at community level and developing a sense of hygiene and cleanliness in individuals is also necessary to ingrain and sustain safe water practices. The best ways to achieve this is to start at grass roots: through community engagement programs involving schools, homes and neighborhoods. Centers of disease control and prevention utilized social marketing techniques in conjunction with community mobilization to achieve major behavior changes pertaining to safe water practices in Kenya and Madagascar.[7] Similar efforts in Pakistan from both public and private sectors with outreach programs can make a difference.

Pakistan has one of the highest typhoid incidence in under 5 year-olds.[8] We also have a huge burden of viral, bacterial and protozoal diarrhea, and mosquito-borne diseases. Preventive measures must also include vaccination against typhoid, rotavirus, and dengue alongside provision of safe drinking water and sanitation.

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ORIGINAL ARTICLE

Study of Urinary Tract Pathogens and Determination of Their Susceptibility to Antibiotics in the Residents of Lahore

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Abstract

Background
Urinary tract infection (UTI) is second most common and a serious medical condition affecting millions people every year. They are the infections of urinary tract i.e. urethra, bladder, urinary ducts and kidneys. Most of these infections are bacterial, particularly gram negative pathogen. We aim this study to identify the common pathogens leading to UTI in our local population and their antibiotic susceptibility.

Methodology
This study was carried out on the residents of different area in Lahore city between Jan and June 2016, and all research work was conducted in The Institute of Molecular Biology and Biotechnology (IMBB), The University of Lahore (UOL). About 250 samples of the all age groups were collected and investigation was made utilizing two main instruments, questionnaire and urine testing to identify microorganisms responsible for urinary tract infections to explore sensitivity patterns of identified microorganisms to certain antibiotics used in the treatment of UTI.

Results
Of all collected samples (n=250) 109 (43.6%) samples showed no growth on culture plates. Approximately 56% (n=141) were positive samples. Among the positive cases gram negative bacterial pathogens were accountable for about 86% of urinary tract infections in contrast to gram positive bacterial pathogens. *E. coli* were major uro-pathogen 39.72% trailed by *Klebsiella spp.* 16% and *Staphylococcus spp.* 12%. The prevalence of *Candida spp.* 20% was also very high in males and females. With respect to the antibiotic sensitivity test, the results revealed that antibiotics like Nitrofurantoin, Piperacillin-Tazobactam and Imipenem for gram negative bacteria and tetracycline against gram positive bacteria, showed a low resistance rate in this study over commonly used antibiotics.

Conclusion
Urine culture is an important diagnostic tool to confirm urinary tract infections. Rules must be put on the consumption of antibiotics to limit their abuse and misuse. Pragmatic antimicrobial choice in management of urinary tract infections should be founded on facts of local occurrence of causal uro-pathogens in addition to corresponding antibiotic sensitivities than on international guiding principle.

Key words
Urinary tract infections, Microorganisms, susceptibility.

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Introduction
Urinary tract infections are chief health problems affected lots of peoples for long time. They rank second of greatest communal sort of infections in the human body.[1] Urinary tract infections occur in both sexes i.e. males and females. Urinary tract infections are instigated by bacterial pathogens which characteristically grow at the neck of urethral duct and move to the urinary bladder. Maximum contagions are initiated by backward mounting of microbes from the fecal flora of anus through the urethral tube to urinary bladder and kidneys particularly in the women that possess a smaller and broader urethral tube.[2] In men, the micro-organisms often invent from the sub prepucial pouch. Urinary tract infections don't at all times cause sign and symptoms, but when they cause they can contain i.e. wanting to pass urine more over and over again and immediately, if only a small number of drops, scorching pain or a 'scalding' feeling when passing urine, urine that look like bright ruddy or cola colored i.e. a mark bloody urine. Other sign and symptoms of urinary tract infections may be a sensation that the urinary bladder is still full after passing urine, cloudy urine, robust odor in urine, pain above the pubic bone in men and pelvic pain in women.

The bacterial strains that cause Urinary tract infections (UTIs) take account of *Escherichia coli* which are accountable for greatest uncomplicated cystitis issues in females, particularly in younger ladies. *Escherichia coli* are commonly an inoffensive microbe originating in guts. If it reaches to the vagina, it can inhabit urinary bladder, creating infections. *Staphylococcus*

saprophyticus are responsible for 5 – 15 percent of Urinary tract infections (UTIs) typically in younger females.[3] The doubtful bacteria *Streptococcus sp*. and *Pseudomonas sp*. are the most frequently causative micro-organisms of urinary UTIs.[4] *Klebsiella, Enterococci* species of bacterial pathogens and *Proteus organism* reason for other bacterial pathogens that responsible for UTIs. They usually found in urinary system in older females. *Lactobacillus, Corynebacterium* and *Streptococcus mileri* probably will be cause of the pathogenesis of urinary tract infections.[5] Rare microorganism causes of urinary tract infections include *ureaplasma urealyticum* and *Mycoplasma hominis* that are usually inoffensive organisms. The objectives of antimicrobial treatment were to eliminate the bacteriological progression in the urinary system using antibiotics that is effective, harmless, and asking price. The tenacity of contagion is reliant on sensitivity of the bacterial pathogens to concentrated amounts of the antibiotics attained in urine.

Material and MethodsStudy settings

This study was carried out on the residents of different area in Lahore and all research work was conducted in The Institute of Molecular Biology and Biotechnology (IMBB), The University of Lahore (UOL).

Sources of data

Over the course of study Patient's information such as age, sex, clinical presentation, existence or nonexistence of urinary catheter was noted. Brief history of patients was gained about UTIs sign and symptoms.

Sample Size

Overall 250 urine samples were collected, about 120 males and 130 females and stayed in the age of 01 to above 50 year old. There were higher numbers of samples collected from the all age groups excluding neonates and infants because of samples collection is much difficult in sterile conditions.

Collection of urine specimen and transportation

The samples were taken into clean, broad necked, leakage resistant, plastic containers. Mid-stream urine specimens were taken from the males and females residing in the Lahore. Each of the people was instructed verbally on mode of collection of mid-stream urine that was during forceful urination after the 10-20 ml has been voided. Participants were adequately educated on precautions to prevent contamination of specimen. All the study participants were fully informed of the purpose of study and consented to provide specimens before starting the study. Urine samples were transported immediately without any prolonged delay in Microbiology laboratory in sterilized conditions.[6]

Sample Preparation and Microscopy

Urine samples were prepared for microscopy according to standard method. The urine specimens were homogenized and aliquots rotated (centrifuged) at 5000 rpm for 5 minutes. The deposited materials were inspected by individually 10X then 40X objective lens. Specimens' with $\geq$10WBC/mm^3 were considered as pyuria.[7] Media used were Nutrient Agar (NA) and CLED Agar as termed by Cheesbrough.[8]

Sample culturing

The collected urine samples were processed for culture and report according to standard method procedure and protocol. For the visual examination and calibrated Loop Streak method for urine culture performed 1 ul wire loop was treated by heat and immersed in urine specimens. Wire loop was at that time streaked on dishes of CLED Agar.[8] Bacteria were reported in colony-forming units (CFU). Less than 10^4/ml organisms were not considered significant, 10^5/ml were significant bacteriuria.

Identification of Uro-pathogens

All the microbial isolated organisms were categorized according to form and structure of colonies, cell morphology**,** Gram stain, Catalase test, and DNase and oxidase tests, Germ tube test. On basis of the respective results of primary identification test the gram negative isolates were exposed to predictable biochemical tests i.e. citrate utilization test, triple sugar iron reaction indole production, urease production, coagulase, Bile solubility, litmus milk decolorization test and motility test.[9] Antibiotic treatment was also performed for GBS.

Pathogen sensitivity test

Entirely isolated micro-organisms were tested against various antibiotics in laboratory via disc diffusion assay method on Muller Hinton Agar by improved Kirby-Bauer method.[10] The zone size and susceptibility testing was don according to CLSI book Performance Standards for Antimicrobial Susceptibility Testing 27th Edition.

Statistical analysis

All data were statistically analyzed by taking due care for completeness, consistency, coding and sorting using SPSS statistical package, version 22.0

Results

Isolated uro-pathogens in male and female participants

The occurrence of urinary tract infections isolated microorganisms in relative to sex is revealed in Table 2. *E. coli* was furthermost recurrently isolated uro-pathogen in both genders though prevalence of *Klebsiella* was higher in males than females. More isolates of *Staphylococcus species* and *Group B Streptococcus* (GBS) were recovered more from females than males. One case of *Neisseria gonorrhea* was positive in males. *Candida* was most prevalent after *E. coli* in both sexes but its percentage is higher in males. There is no prominent sex difference for the remaining isolates i.e. *Proteus, Pseudomonas* and *Citrobector.*

Prevalence of Gram positive and negative isolates of UTIs

Most of the cases of urinary tract infections were because of

Table 1. Isolated uropathogens in male and female participants

Organisms Isolated	Males	(n,%)	Females	(n,%)
E. coli	37	(50%)	19	(28%)
Klebsiella	13	(18%)	10	(15%)
Staphylococcus aureus	03	(4%)	06	(9%)
Staphylococcus epidermidis	03	(4%)	03	(5%)
Staphylococcus saprophyticus	01	(1%)	01	(1%)
Pseudomonas	03	(4%)	02	(3%)
Proteus	01	(1%)	01	(1%)
Group B Streptococcus (GBS)	02	(3%)	04	(6%)
Neisseria gonorrhea	01	(1%)	00	(0%)
Citrobector	01	(1%)	01	(1%)
Candida	09	(12%)	20	(30%)
Total	74	(100%)	67	(100%)

gram negative *bacilli* 85.93 percent. Gram negative *bacilli* cases were higher in males than females. However the gram positive *cocci* were less prevalent than gram negative *bacilli.* The prevalence of gram positive *cocci* was higher female than males.

Percentage susceptibility in Gram negative uro-pathogens
Isolated organisms for *Escherichia coli* presented resistance to frequently used antimicrobials. The highest sensitivity was shown by Gentamicin, Imipenem, Cefotaxime and Nitrofurantoin. *Klebsiella* isolates were only sensitive to Imipenum. *Pseudomonas* was sensitive to Amikacin, Cefoperazone, Piperacillin-tazobactam. *Proteus* isolates were highly sensitive to Cefotaxime, Tazocin, and Cefoperazone. Isolates of *Citrobacteria* were sensitive to orally administered antibiotics Amoxicillin-Clavulanic acid and Nitrofurantoin. It was also sensitive to injectable antibiotic Imipenem. *N. gonorrhea* was not sensitive to any tested antibiotic.

Percentage susceptibility in Gram positive uro-pathogens
Staphylococcus aureus was among highly susceptible to ciprofloxacin, tetracycline and Gentamicin. *Staphylococcus epidermydous* was 100% sensitive to Novobiocin and highly susceptible to Nitrofuratoin. *Staphylococcus saprophyticus* was sensitive to Cefotaxime. *Group B Streptococcus* (GBS*)* showed high susceptibility to Tetracycline, Nitrofuratoin and Novobiocine.

Discussion
Urinary tract infections mean different types of medical illnesses extending in sternness from symptomatic to asymptomatic renal infection with resulting sepsis.[11]

The uro-pathogens recognized in our research are alike to numerous readings conducted in different parts of the world either in local area or universally. Pathological microbes were isolated from 141 positive samples which in the vast majority of cases were of *E. coli* (39.72%) followed in order by *Candida* fungus (20.57%), *Klebsiella* (16.31%) *staphylococcus* (12.06 %), *Group B Streptococcus* (4.25 %), *Pseudomonas* (3.55 %), *Proteus* (1.42 %), *Citrobector* (1.42%) and *Neisseria gonorrhea* (0.71%) were among the most common urinary pathogenic bacteria. That was as to other readings where it places the most common uro-pathogen causing urinary tract infections as studied by Mehar where 62.6% cultures grew *E. coli*.[12] These consequences remained also alike that a research directed by Dilnawaz in 2005 in India that showed first two common uro-pathogens was *Escherichia coli* and *Klebsiella pneumonia;* both microorganisms were different with respect individual percentage from previous studies.

The prevalence of the urinary tract infections pathogens in relative to sex is presented in Table 2 and 3 are steady with reports from Abubakar.[13] Most of the urinary tract infection cases were because of gram negative *bacilli* 85.93 percent. Gram negative *bacilli* cases were higher in males than females. However the gram positive *cocc*i were less prevalent than gram negative *bacilli.* The prevalence of gram positive *cocci* was

Table: 2. Percentage susceptibility in Gram negative uro-pathogens

Organisms	Antibiotics									
	AML	AK	CIP	CTX	CPZ	GEN	IPM	NA	NIT	PTZ
E. coli	20	52	28	70.0	10	90	88	35	77	69
Klebsiella	21	65	15	68	35	20	90	25	61	66
Pseudomonasaeruginosa	45	85	11	NA	75	69	57	5	26	81
Proteus	61	78	31	95	84	63	75	65	59	85
Citrobacteria	74	56	19	53	44	59	73	24	89	55
N. gonorrhea	65	20	12	14	16	22	69	25	20	58

E: Escherichia, N: Neisseria

AML-Amoxicillin-Clavulanic acid, AK-Amikacin, CIP-Ciprofloxacin, CTX-Cefotaxime, CPZ-Cefoperazone, GEN-Gentamicin, IPM-Imipenum, NA-Nalidixic, NIT-Nitrofuratoin, PTZ-Piperacillin-Tazobactam.

Table 3. Percentage susceptibility in Gram positive uro-pathogens

Organisms	Antibiotics										
	AML	CIP	CTX	CPZ	E	GEN	NA	NIT	P	TC	NV
S. aureus	63	76	58	54	48	80	35	63	29	77	67
S. epidermydous	29	18	52	41	56	19	44	83	24	67	100
GBS	38	18	8	NA	48	12	15	71	00.0	95	97

S: Staphylococcus, GBS: Group B Streptococcus
AML-amoxicillin-Clavulanic acid, CIP-Ciprofloxacin, CTX-Cefotaxime, CPZ-Cefoperazone, GEN-Gentamicin, E-Erythromycin, NA-Nalidixic, NIT-Nitrofurantoin, P-Penicillin, TC-Tetracycline, NV-Novobiocine.

higher female than males.
Consequently that higher prevalence of *E. coli* might be due to fecal contamination, the predilection of the organisms from the toilets and the shortness of the female urethra in females. That occurrence was also reported in previous research don by Australian Smith[6]. There is a conceivable relation among UTIs prevalence amongst the population and the standard of personal hygiene and the condition of toilet facilities. It is previously reported by Fihn.[14]

Many people inspected valued the toilets were bad. Bad, in that situation suggests that there was no suitable water supply for cleaning and flushing toilets on public places congestive areas frequently. According to this case population were at the risk of becoming infected during passing urine, that was observed by visiting different areas in Lahore and through by asking questions from population. Sexual activity was another factor that predisposes people to UTIs. For example, *Staphylococcus aureus* (12.06 %), which was a member of skin flora might stay on the skin and get transmitted during sexual intercourse. If demographic situation of the cases examined by age then it was observed that both the extremes of age groups have risk of UTIs. These results are similar to Handley.[15] There were many cases of UTIs caused by *candida spp.* (20.57%). *Candida* urinary tract infection was typically presented in the patients with immune-suppressant.[7] These results of antibiotic sensitivity shown in Tables 4 and 5 resemble to the research of Dr. Alka Nerurkar.[16]

The implication of that was the opportunity of easy access of self-medication, exploitation of medicine leading progress to resistance against pathogens. It is recommended to establish good management of drugs in order to prevent their easy accessibility in public sector without recommendation. Public awareness about the potential risk factors of UTIs is important to reduce the risk of the disease. In this study, all participants of different age groups and children above one year were included, where as participants with known T.B, on urinary catheters and children below one year old were excluded.

Conclusion

There is an emerging resistance of commonly isolated bacteria to commonly used antibiotics, which can be ascribed to inappropriate antibiotic administration. Pragmatic antibiotic choice in dealing of urinary tract infections must be grounded on the information of indigenous prevalence of contributing uro-pathogens and their respective antibiotic sensitivities rather than on universal guidelines. *Candida* was most prevalent after *E. coli* in both sexes but their percentage is higher in males because female cases do not report well to hospital due to ethical issues in under study area. Undiscriminating recommendation and usage of antibiotics necessity be disheartened in both public and clinical trials by incessant public alertness and teaching on coherent antimicrobials usage and their choice of antimicrobials must be on the basis of culture and sensitivity test.

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ORIGINAL ARTICLE

Compliance with the Sepsis Resuscitation Care Bundles in the Emergency Department of a Developing World Tertiary Care Hospital – A Clinical Audit

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Abstract

Background
Severe sepsis and septic shock are catastrophic syndromes resulting in a systemic inflammatory response and dysfunction of one or more end organs. The Surviving Sepsis Campaign is an international collaboration in order to reduce mortality in severe sepsis and septic shock using a standard bundle of care approach.

Method
We conducted a retrospective observational clinical audit in the Department of Emergency Medicine at the Aga Khan University Hospital, Karachi. The compliance rate against each element of the 3 and 6 hour bundle were obtained.

Result
Majority of patients were female with mean age of 64 years. Blood lactate was undertaken 46.5% of the time however majority of patients received timely intravenous fluids and antibiotics. Blood for Culture and sensitivity and blood lactate clearance were measured poorly.

Conclusion
We conclude that compliance to sepsis resuscitation bundles in our setting is inadequate therefore higher training, education and increased awareness is imperative.

Keywords
Sepsis; Surviving Sepsis Campaign; Bundle; Compliance

Introduction

It is estimated, that globally, 5.3 million people die of sepsis on an annual basis.[1] The current burden of sepsis mortality is mostly gestating in developing world- countries which are least able to bear it. At least 50% of septic patients in low-income countries are reported to die, compared to a mortality rate of 15-30% in the United States.[2] Asghar *et al*. demonstrated that there was a high incidence of sepsis in patients admitted to the Intensive care unit of a teaching hospital in Karachi associated with a very high mortality of 51%.[3]

In year 2002, efforts led by the Surviving Sepsis Campaign (SSC) to reduce sepsis-related mortality were intensified.[4] To this end the SSC developed an evidence-based guideline which was distilled into precise bundles of care.[5] The effectiveness of the sepsis bundles lies in the timely achievement of each bundle element. In 2014 Levy *et al* demonstrated that a 10% increase in compliance with the SSC bundles reduced in-hospital mortality by 3-5%. In addition, for every 3 months that a center complied with the SSC bundles, in-hospital mortality declined by 7%.[6] Numerous studies over the past years have clearly demonstrated that improved compliance with the SSC bundles is associated with shorter hospital and ICU length of stay, as well as reduced mortality.[7-10] Ironically, it is the developed world from which most research on sepsis originates. According to a meta-analysis conducted in 2016,[1] population-level epidemiologic data for sepsis are scarce to nonexistent for low-and middle-income countries. Since the advent of the Surviving Sepsis Guidelines in 2004, few studies to assess compliance with the guidelines in the developing world have been conducted. To date, the MOSAICS study (Management of Severe Sepsis in patients admitted to Asian Intensive Care Units, 2011) is the largest multicenter study to prospectively assess compliance to the SSC bundles in the developing world. From the 150 participating ICUs in 16 Asian Countries, compliance rates of 7.6% and 3.5% were observed for the resuscitation and management bundles respectively.[11]

Optimization of Emergency department (ED) management of the septic patient is a priority, as prior studies have shown that two-thirds of septic patients arrive in the ED.[12,13] We are currently lacking studies conducted in our local setting for evaluating the compliance with SSC bundles in ED. Therefore we conducted a clinical audit to determine the compliance to various elements of the 3 and 6 hour SSC bundles for adult patients with severe sepsis and/or septic shock presenting to the ED.

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Methodology

We conducted a retrospective observational clinical audit in the Department of Emergency Medicine at the Aga Khan University Hospital (AKUH), Karachi, Pakistan over a period of 6 months starting from January till June 2017. Due to the retrospective nature of the audit, we were granted exemption from informed consent by the Ethical Review Committee (ERC).

Severe sepsis and septic shock were diagnosed as per the current Society of Critical Care Medicine (SCCM) guidelines.[4] Severe sepsis was defined as sepsis with signs of acute organ dysfunction or hypoperfusion indicated through; sepsis-induced hypotension (systolic blood pressure (SBP) < 90 mm Hg or mean arterial pressure (MAP) < 70 mm Hg or a SBP decrease > 40 mm Hg or less than two standard deviations below normal for age in the absence of other causes of hypotension), blood lactate above upper limits laboratory normal (> 4 mmol/L), urine output < 0.5 mL/kg/hr for more than 2 hours despite adequate fluid resuscitation, acute lung injury with PaO2/FIO2 < 250 in the absence of pneumonia as infection source, acute lung injury with PaO2/FIO2 < 200 in the presence of pneumonia as infection source, serum creatinine > 2.0 mg/dL, total bilirubin > 2 mg/dl, platelet count < 100,000 µL, coagulopathy (international normalized ratio > 1.5). Septic shock was defined as severe sepsis in the presence of hypotension (systolic blood pressure <90 mm Hg) refractory to adequate fluid resuscitation (administration of IV Fluids 30ml/kg over initial 3 hour duration).

In view of a recent study,[3] 99 patients were identified having sepsis with an incidence of severe sepsis and septic shock of 81%. Utilizing existing data and considering confidence intervals at 95 %, sample size was calculated to be of 71 cases.

Prior to data collection, the principal investigator conducted a session to explain the study protocol and to train the data collectors in filling the questionnaire. The data collectors searched the Electronic medical record database for adult patient encounters in ED bearing an ICD9 code diagnosis of 995.92 and 785.52 for severe sepsis and septic shock respectively. We adopted a non-probability sampling strategy for including adult subjects (age ≥18 years) with a diagnosis of severe sepsis/septic shock upon admission to the ED of Aga Khan University Hospital (AKUH). Subjects were excluded if they were unable to survive for 6 hours duration after recruitment (independent of timely institution of the sepsis resuscitation bundle), Do Not Resuscitate (DNR) or Left Against Medical Advice (LAMA) within 6 hours of triage, resuscitated at a different hospital prior to transfer to the ED or had incomplete medical records. The confidential files of these patients were reviewed in order to look for fulfillment of criterion for Severe Sepsis and Septic Shock and the compliance to various elements of the Surviving Sepsis Campaign 3 and 6 hour bundles. The 3 hour bundle tasks included; measuring serum lactate level, obtaining blood cultures (before starting antibiotics), starting broad spectrum antibiotics, and administering a 30 mL/kg crystalloid bolus for patients who are hypotensive or have an elevated serum lactate or signs of organ hypoperfusion. The 6 hour bundle tasks included; starting vasopressors for hypotension that is refractory to volume resuscitation to maintain a mean arterial pressure greater than 65 mm Hg, reassessing intravascular volume status, and remeasuring lactate after 3 hours of resuscitation and if the initial lactate was > 4 mmol/L.

SPSS-version 19 was used for statistical data analysis. In descriptive variables, the continuous variables were displayed using mean and standard deviation while the categorical variables were displayed using percentages (proportions).

Results

A total of 71 patients were included in the study with a mean age of 64 years. Out of these patients, 42 (59%) patients were female and 29 (40%) patients were male. 19 (26.8%) patients were diagnosed as having severe sepsis and 52 (73.2%) patients had septic shock while in the ED. 35 (49%) patients were admitted to the Intensive care unit**.**

When we reviewed compliance with each element of sepsis resuscitation bundles the following results emerged. In the initial 3 hour bundle, 48 (67.6%) patients received antibiotics within 3 hour duration. Only 4 (5.6%) patients had blood drawn for Culture and sensitivity (C/S) prior to antibiotic initiation. Serum lactate testing was performed in 33 (46.5%) patients only. Intravenous fluids administration by 30 ml/kg was done in 60 (84.5%) patients resulting in adequate fluid responsiveness in 43 (60.6%) patients.

In the subsequent 6 hour sepsis resuscitation bundle, Vasopressors were applied to 35 (49.3%) fluid refractory hypotensive patients. Central Venous line insertion was performed and measured in 41 (57.7%) patients. A cutoff CVP pressure of > 8 mmHg was able to be established in 20 (28.1%) patients. The Central Venous Oxygen saturation (ScvO2) was measured in 9 (12.7%) patient and a target ScVO2 of >/= 70% was achieved in 2 patients only. 13 (18.3%) patients had their lactate levels remeasured for successful lactate clearance**.**

Discussion

The Sepsis resuscitation bundles provide a standard operating procedure for early risk stratification and management of a patient with severe infection. Application of the bundle resulting in a clinical and statistically significant decrease in organ failure, mortality, and the utilization of health care resources has been successfully demonstrated.[14,15] However, quality improvement initiatives undertaken in emerging countries have faced various challenges.[16]

Our audit demonstrated that patients received Intravenous fluids and antibiotics in timely manner as emphasized in published guidelines[18]. Nearly 50% of our patients who were hypotensive after fluid administration were started on vasopressors and CVP

line insertion was instituted in 60% of patients. However, post CVP management was lacking in its entirety.

The measurement of lactate levels has been associated with improved outcomes in sepsis, and an elevated lactate value identifies patients at higher risk for poor outcomes.[17] In the first quarter of a multicenter quality improvement program for sepsis care, only 61% of patients had lactate values measured consistent with guidelines.[18] In addition, prior studies have shown that care prompted by measurement of lactate levels in sepsis patients reduces resource utilization and cost and leads to lower likelihood of hospital-acquired conditions.[19] Our audit analysis showed initial lactate testing performed in 46.5% patients. Elevated lactate levels prompt the consideration of specific care practices toward hemodynamic optimization guided by either central venous oxygen saturation[12] or lactate clearance.[20] Performance gaps in ScvO2 measures can be as low as 15% as observed in community EDs to 50% in larger tertiary care centers[21] compared to 13% observed in our setting. International guidelines[14] now strongly recommend that septic patients with elevated lactates have additional therapies until lactate levels are normalized however, only 18 % of our patients were measured for lactate clearance.

Collecting blood cultures has been specifically associated with improved outcomes in sepsis. By obtaining blood cultures, antibiotic regimens can be customized to treat the specific infecting organism. This will result in less unneeded exposure to antibiotics, reducing complications associated with antibiotic use, including drug reactions, allergies and adverse events and the development of drug-resistant organisms.[14] Prior studies have shown only 64.5% of patients had blood cultures collected.[18] In our analysis, only 5.6% patients had blood drawn for cultures prior to antibiotic initiation.

Difficulties in implementing sepsis protocols have already been reported and include a lack of dedicated staff,unavailable resources,[22] shortage of medical and nursing staff,[23] and reduced compliance with the basic principles of quality care, such as continuous training strategies which is associated with a high professional turnover rate,[24] contributing to an inadequate safety culture and low quality of care.[25] These institutional characteristics were not addressed in our study. Also, we did not assess other institutional factors that might be associated with higher sepsis mortality rates, such as the availability of ICU beds or the percentage of patients transferred from other facilities.

Conclusion

The audit has demonstrated that compliance to sepsis resuscitation bundles in our setting is inadequate therefore higher training, education and increased awareness is imperative. Given current and existing evidence, greater compliance with each element of the bundles will result in better patient outcomes compared to what is being observed under present circumstances.

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Risk Factors and Outcome Differences in Methicillin Resistant and Methicillin Sensitive *Staphylococcus Aureus* Septic Arthritis

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Abstract

Background
Septic arthritis caused by *Staphylococcus* is very common and causes high mortality and economic burden. In addition, rising cases of its resistant strains (Methicillin resistant *Staphylococcus Aureus*) is alarming. Conflicting results in literature mandates a comparison between resistant and sensitive strains in terms of risk factors, mortality and morbidity which becomes crucial in this regard to delineate any differences in our population.

Results
We gathered 66 patients with septic arthritis, out of which 46 were men, with mean age (SD) of 51.4(18.9) years. 30 patients had MRSA and 36 had MSSA. Most patients (n=61) had one joint involved, the most common joint was the knee joint (n=31), followed by hip (n=21), ankle (n=11), shoulder (n=4) and elbow (n=2). Most patients(49) had native joints involved. The inflammatory markers were not significantly different across the two groups. Hospital stay in both groups was almost similar(9.6 days for MRSA and 10.3 days for MSSA). Mortality3(10.0) was higher in MRSA group.

Conclusion
No differences were found in the inflammatory markers as well as synovial fluid and blood white cell count between patients infected with Methicillin resistant and sensitive *Staphylococcus aureus* these should not be used as a differentiating factor between the two.

Keywords
MRSA, MSSA, Septic arthritis, risk factors.

Background
Septic arthritis is a fulminant infection of joints which impacts 2 to 10 per 10,000 patients on a yearly basis and impairs joint function in 25 to 50% cases.[1] it causes significant debilitating morbidity and mortality if not treated timely and leads to excessive financial burden.[2,3,4,5] The commonest pathogen involved in septic arthritis is *Staphylococcus aureus*[3,6,7] which is a virulent organism causing a cataclysmic mortality of 15% if not treated emergently with appropriate medical and surgical management.[6,7,8,9] Staphylococcal infections have been known to cause immense economic burden with methicillin resistance causing steep health costs.[3,10,11]

Methicillin resistant *Staphylococcus aureus* (MRSA) has emerged as an established nosocomial pathogen all over the world.[6,12] There have been several western and a couple Asian studies highlighting the importance of methicillin resistant *Staphylococcus aureus* (MRSA) septic arthritis in terms of escalating virulence, relationship with underlying comorbidities and risk factors and the age group affected in comparison to methicillin sensitive strains (MSSA).[8,9,13] However there have also been conflicting studies indicating Methicillin sensitive *Staphylococcus aureus* (MSSA) to have an equal pathogenicity.[14] Data from the subcontinent is however lacking in this regard. A comparison between MSSA and MRSA septic arthritis in terms of mortality, risk factors and their association with length of hospital stay becomes crucial in our population as MRSA is deemed a difficult to treat infection on account of resistance to a major class of antibiotics and limited options of antibacterials available.[15]

Initially thought of as a hospital acquired pathogen research has revealed an upscale surge in MRSA acquired from community settings called community acquired MRSA which is another reason to study this in patients coming with septic arthritis.[16]

Inequalities in health conditions are well established in different populations of the world in terms of social income, ethnic, genetic and demographic differences. Pakistan being a low income country with all the aforementioned differences in its population as compared to the west is more prone to long standing ailments and comorbidities and these figures are on the rise as stated by a report. Pakistani population are more prone to Cardiovascular diseases and diabetes mellitus almost thrice as compared to the west.[17] Also low income countries have increased risk of infections with infections being a major

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cause of death as compared to high income countries.[18] On account of these differences, it is of unrelenting importance that we study the difference in outcomes as well as risk factors and prevalence in our patients and our population and do risk profiling between MSSA and MRSA.

There have been studies indicating that MRSA bacteremia and arthritis is associated more with risk factors like hemodialysis, malignancy, intravenous line usage, increased hospital stay, old age, as compared to MSSA arthritis,[19,20] but there have not been any studies in our population setting which is entirely different than the western and Asian population. The objectives of conducting this study were to determine the difference in risk factors, mortality and length of hospital stay between MRSA and MSSA septic arthritis.

Materials and Methods

A retrospective cross-sectional study was conducted. Patients with either MRSA or MSSA positive identified in pus culture from the joint or blood (with joint involvement) presenting to AKUH during 2000 to 2015 were identified from the medical records and their files were reviewed.

All cases of MRSA and MSSA septic arthritis were screened for the selection criteria that is adults aged 18 to 85 years diagnosed with septic arthritis as per radiological or clinical signs of septic arthritis with pus cultures or blood cultures growing *staphylococcus aureus*. Patients with mixed growth that is *Staphylococcus Aureus* and other organisms were not included.

Statistical analysis was conducted using statistical package for social sciences (SPSS, version 19.0). Mean with standard deviation were reported for all quantitative variables such as age, duration of hospitalization, number of joints, WBC count, clearance days, ESR, CRP etc. Frequencies with percentages were reported for all categorical variables such as gender, comorbid conditions, type of malignancies, type of joints, use of antibiotics and outcomes. Independent sample T-test was used to compare quantitative variables across the categories of MSSA and MRSA arthritis and Chi square test was done to compare categorical variables. P-values of <0.05 was considered to be significant.

Results

During the study period, 66 cases of *Staphylococcus Aureus* were identified from the records with mean age (SD) of 51.4 (18.9) years, half of the patients (50%) were aged 55 and above. Majority patients were men (n=46, 69.7%). Clinical characteristics of study patients are given in table 1. Most common comorbid condition was hypertension (30.3%) followed by Diabetes (22.7%) and osteoarthritis (16.7%).About 9 patients had chronic kidney disease,8 had a history of trauma, 6 were on steroids, 5 had chronic liver disease, 5 were dialysis dependent and 4 patients had malignancy, one each of Giant cell tumor, B cell lymphoma, Spindle cell tumor and T cell lymphoma. In addition, 3 had pulmonary Tuberculosis and 1 had Asthma. Most patients had single joint involved (n=61, 92.4%), followed by two (n=4, 6.1%) and three joints (n=1, 1.5%). About 49 patients had Native joint and 16 had prosthetic joint involvement. Knee was the commonest joint involved (n=31, 46.9%), followed by hip (n=21,31.8%), ankle (n=11,16.6%), shoulder (n=4, 6.0%) and elbow (n=2,3.0%).

Table 1 also shows comparison between patients with MRSA and those with MSSA. Patients with MRSA were older (53.5 vs. 49.6) as compared to MSSA. Immunosuppressive drugs, transplant, trauma, hemodialysis and Chronic kidney disease (CKD) were found to be more common with MRSA (p value =0.28), however, Chronic liver disease (CLD), Diabetes Mellitus (DM), Hypertension and central line placement showed no difference amongst both the groups. The inflammatory markers and WBC were not different across the two categories. However, synovial fluid WBC were higher for MRSA group. There was no difference in hospital stay in both groups (9.6 days MRSA 10.3 days MSSA). The duration between discharge and readmission was shorter for MRSA as compared to MSSA (3.47 ± 7.8 vs. 7.5 ± 21.5). Comparison of joints involved across both the infections are presented in figure 1.

Three of the patients with MRSA died and 1 was lost to follow-up. In contrast, there were no deaths in MSSA group. Statistically significant differences were seen across the categories of blood and pus fluid cultures. MSSA was isolated more in pus cultures (34 vs. 22) whereas isolation in blood was higher for MRSA (15 in MRSA and 11 MSSA). Clearance from blood took significantly more time in MRSA (68.5 days) as compared to MSSA (14.0 days).

Discussion

Extensive literature search shows results slightly similar to our study. A retrospective 13 year study from West Texas Shows 22.6 % of septic arthritis to be MRSA with a mortality of 5.5% with a length of hospital stay of around a fortnight to a month. The elderly, MRSA infection and prosthetic joint infections were associated with worse outcomes.[21] A study found MRSA to be more than half of MSSA cases and more prevalent in IV drug abusers (IVDU).[22] Contrastingly, Al-Nammari states that MSSA is seen more in IV Drug users which co relates with our study results of finding more MSSA in central line users, also knee involvement was more seen with MSSA.[8] Another study with 93 Staphylococcus septic arthritis cases found 38 (40.9%) patients with MRSA out of which around 90% were deemed community-acquired. Also they found MRSA to be more associated with Diabetes mellitus (44.1%), chronic kidney disease and liver cirrhosis. 5.4% was the mortality rate, this contrasts with our results.[23] A study looking at the knee joint only found *Staphylococcus* to be the dominant bacteria with 72.1% and mostly isolated from synovial fluid cultures with isolation on gram stain in around half of the cases.[24] A 5 year

Table1: Comparison of characteristics between patients with MRSA versus MSSA

Characteristics	Overall	MRSA n=30 n(%)	MSSA n=36 n(%)	P-Values
Age, mean(SD), range: 17-87	51.4(18.9)	53.5(19.6)	49.6(18.4)	0.40
Gender				
Male	46(69.7)	21(70.0)	25(69.4)	0.96
Female	20(30.3)	9(30.0)	11(30.6)	
Previous hospitalization, mean(SD), range: 0-120 days back n=13	5.67 (16.7)	3.47(7.8)	7.5(21.5)	0.33
Duration of hospitalization, mean(SD), range: 3 to 27 days	10.05(6.05)	9.6(5.3)	10.3(6.6)	0.64
Number of joints, mean(SD)	1.09(0.33)	1.07(0.25)	1.11(0.39)	0.58
Type of Joint				
Native	49(74.2)	16(24.2)	1(1.5)	0.61
Prosthetic	22(73.3)	8(26.7)	0(0)	
both	27(75.0)	8(22.2)	1(2.8)	
Culture and Sensitivity Blood	26(39.3)	15(50)	11(30.5)	<0.001
Culture and Sensitivity Pus Fluid	56(84.8)	22(73.3)	34(94.4)	<0.001
Clearance Days, mean(SD), range: 2-304 n=19	42.6(92.0)	68.5(123.4)	14.0(13.2)	0.20
ESR, mean(SD), range: 10-134 n=46	79.07(33.6)	84.6(32.1)	75.1(34.7)	0.34
CRP, mean(SD) range: 0.1-40.9 n=52	14.2(10.7)	11.1(7.0)	16.8(12.6)	0.05
WBC, mean(SD), range: 1.7-47.5	13.8(8.6)	14.5(10.7)	13.2(6.4)	0.57
Fluid WBC, mean(SD), range: 4000 to 143,800	39151.67	39460(47714.3)	38931(47895.2)	0.98
Outcomes				
Alive	62(93.9)	3(4.5)	1(1.5)	0.07
Dead	26(86.7)	3(10.0)	1(3.3)	
Lost to follow	36(100)	0	0	

SD, Standard deviation; ESR, Erythrocyte sedimentation rate; WBC, white blood cell; CRP, C-reactive protein

spanning retrospective study published in 2007 found 58 cases of staphylococcus septic arthritis involving only 15 MRSA and 43 MSSA with MRSA infecting the 70 plus population mostly. Ross *et al* found a significant association between prior hospitalization and MRSA (80% cases compared with 34% in MSSA) which our study failed to identify, also the mean number of comorbidities were higher in MRSA in their study and specifically involving the elderly. No significant difference was

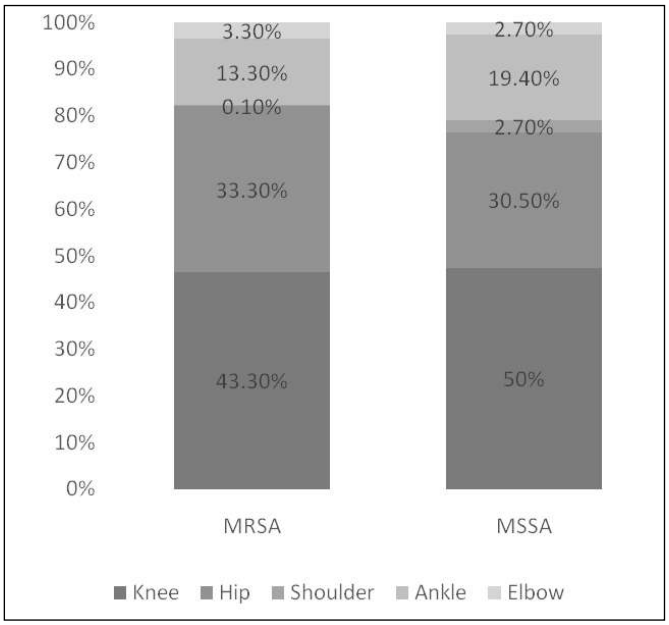

Fig 1. Comparison of joints involved in MRSA versus MSSA Septic arthritis

found in the type of joint involved however, except that shoulder involvement was more common in MRSA (40% vs.14%).[19]

Conclusion

MRSA is more prevalent in patients on immunosuppressive, Dialysis dependent and kidney failure with higher mortality rates in MRSA, therefore this should be kept in mind and Vancomycin containing regimens must be chosen empirically till cultures finalized. Also no difference was found in the inflammatory markers and total leukocyte count in blood among the two groups so these should not be used as a differentiating parameter.

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ORIGINAL ARTICLE

Healthcare Providers' Knowledge and Frequency of Healthcare-associated Infections in Secondary Level Hospitals of Karachi

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Abstract

Background
Healthcare Acquired Infections (HAIs) are significant public health challenge in world. HAIs are acquired during the course of treatment in a healthcare setting. Infection control measures can decrease HAIs by more than 50%.

Methods
A cross sectional study was carried out in five secondary level hospital supervised by a Karachi based NGO. Data was collected in two months March-April 2017.A total of 350 healthcare workers were randomly selected from the 5 hospitals and analyzed in the final data. Data was analyzed using IBM SPSS Version 21.

Association between categorical variables were assessed through the application of Chi square and difference in knowledge scores among different hospitals employees identified through their numbers of beds and was taken out by the application of ANOVA. Significant valueis less than 0.05.

A total of 400 questionnaires were distributed out of which a total of 350 were answered, giving the response rate of 87.5%. Significant difference was observed among the hospitals (F 2.698 P Value 0.03) when preparedness against infection was assessed. 22 bedded hospital was found to have a higher preparedness score among all the hospitals. Maximum frequency of nosocomial infections was seen in hospital with maximum number of beds.

Conclusion
This study indicated that the current status of nurse knowledge related to Nosocomial infections was poor. Policies on infection control and training and re-training of HCW are highly recommended.

Key words
HAI, Nosocomial infection, Healthcare worker, Infection control measure, Secondary level hospital.

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Introduction

Nosocomial or Healthcare-associated infections (HAIs) are a serious public health challenge worldwide affecting both developed and developing countries.[1] Healthcare-associated infections are systemic or localized condition that results from adverse reaction of an infectious agent or its toxin that was present 48 hours or more after admission in a hospital and not incubating at admission time.[2] Nosocomial infections are one of important causes of serious illness and even death in critically ill patients. Out of every 100 hospital admissions 7 patients in developed and 10 in developing countries are victim of Hospital Acquired Infections. Healthcare-associated infections imposes high cost on individuals, societies, states and health care institutions.[3] Large proportion of Nosocomial infections can be prevented and they are considered as an indicator of quality of health care system.[4] Infection control practices can reduces HAI by more than 50 %.[2] These infections are usually spread by health care workers that have poor compliance with infection control measures especially poor hand hygiene.[5] These infections account for an estimated 90,000 preventable deaths per year.[5] Large number of Healthcare-associated infections result in prolongs stays in hospitals, delayed patient recovery and may results in patient's death. The additionalcosts of disease arebearded primarily by patients and their families, hospitals and by society as a whole.[7-9]

The prevalence of Nosocomial infections is now increasing in world and becoming a challenge for health care professional. In developing countries like Pakistan, these infections cause financial burden overpatients and health care institutions. Health care system of Pakistan is not well developed, health budget is very low and nurse to patient's ratio is not according to standard. Medical insurance system is not existed in Pakistan and most of patients pay expenses by themselves or by state in government run hospital. Prevention and control of HAIs requires the presence of policy and organization, health care worker training, defined protocol for cleaning of operation room, delivery rooms and hand hygiene, proper sterilization of medical equipment and linen, proper waste disposal, proper use of antibiotics, presence of systems for isolation of patients with infectious diseases and existence of a vaccination program for staff.[10]

Methodology

A cross sectional survey was carried out in five secondary level hospitals supervised by a Karachi based NGO. Data was collected in two month period from March-April 2017. The target population comprised of healthcare providers of five hospital men and women aged 20 or older with more than 6 month of work experience excluding administrative staff. The participant fall into any of following educational categories: Physician includes residents (2 years' experience in respective field). Consultants (Post-graduation in respective field). Nursing staff included Aid Nurse (two years working experience), Midwife (certified midwifery), NICU, OT, Lab, blood bank Technicians (certified technician in respective field), Registered Nurse holding an active RN license. Housekeeping staff i.e. Ward boys and maids (Non Matric but can read and write). Sanitary staff (sweeper and sweepers) illiterate. 400 employees (n=400) were randomly selected from the hospitals roster. Out of 400 employees 350 responded. Employees less than 6 months experience and who did not complete the questionnaire were not included in the study.

Survey tool is a validated questionnaire. The questionnaire was constructed from emergent theme reviewed in literature by following authors De-Oliviera *et al.*[11] ; Edwards *et al* 2009.[12]; Ribby *et al* 2005.[13]; Pittet *et al* 2009.[14]; Pessoa-Silva *et al* 2007.[15]; Sex *et al* 2007.[16] Participant respond were rated on a Likert scale.[17]

The questionnaire consists of three sections, namely demographic features, Health facilities preparedness on infection control and Knowledge of infection control.

Three data collectors and one nurse from each health facility were selected and trained. Illiterate employee who could not read the questionnaire, were facilitated by translator. The data was analyzed using SPSS version 21. Ethical clearance taken from ethical review committee of Health services of NGO. The respondents were informed about purpose of study. Verbal consent was also obtained from study participants. All information gained during data collection was confidential. Data was analyzed using IBM SPSS Version 21. Association between categorical variable were assessed through application of Chi square and difference in knowledge score among different hospital identified through their beds was taken out through the application of ANOVA. P value less than 0.05 was taken as significant.

Results

This cross-sectional study investigated Knowledge of health care providers with regard to spread of Hospital Associated infections. The predictive relationship exists between organizational support and the level of healthcare providers' knowledge.

Demographics Features

350 out of 400 questionnaires were responded, giving the response rate of 87.5%. Proportions of female were n=201 (57.4%) and n=149 (42.6%) are male. Most of the participant lies between the age 21-30 years (35. 1%). 13.1 % participants were greater than 50 years and 6.3% were less than 20 years age.

Regarding the education of participant n=97 (27.7%) were midwifes with one and half year education and only n=31(8.9%) are Registered Nurse with 3 years education. Among the doctors 10.6% were resident and 9.15 % are consultant physician. In term of field experience of participant 62.6 % (n=219) have more than 3 years of experience in fields. In term of employment, majority of participant 32.0% work in maternity ward and 60% work for 6 hours. As far as interest in education of infection control is concerned, 83.4 % of participants have attended workshops and only 12.6% not attended any workshop.

Knowledge of the healthcare employees regarding hospital preparedness for Infection control was assessed on different parameters (Table 1). Regarding hospital Infection Control team, 15 bedded hospital employees n=38 (28%) affirmed that they have an Infection Control team. In 22 bedded hospital n=29(22%) confirmed the presence of team whereas others hospitals employees were found to be less aware of the presence of such team.

60 beds hospital employees n=21(27%) showed that Infection control practitioner is present during every shift while n=19 (24%) HCW of 22 beds hospital was also aware of Infection Control practitioner presence in hospital.

n=31(26%) HCW of 22 bedded hospitals think that improvement of hand hygiene adherence is institutional priority. Equal number of HCW of 25 beds and 10 beds hospital n=27(22%) were also think so.

n=41(26%) health provider of 10 beds hospitals said that their facility had hand disinfectants for patients in ward and in patient's room whereas n=38(24%) healthcare providers of 60 beds gave the same response.

Regarding the availability of disinfectant outside patient's rooms and in corridors n=37(25%) of 22 beds hospital health care worker were agreed with this whereas n=33(22%) of 10 beds employees affirmed that they have hand disinfectant outside patient's rooms and in corridors.

n=36(30%) health care workers of 22 beds hospital affirmed that they are provided with disposable paper towel for staff whereas n=23(19%) employees of 25 beds hospital responded that they are provided with such facility. When enquire about the availability of disposable paper towel. n=30(30%) employees of 15 beds hospital affirmed whereas n=27(27%) health workers of 25 beds hospital have such knowledge. Other hospitals have lesser knowledge of this.

Table 1: Hospital preparedness regarding control of Nosocomial

#	Hospital preparedness	Response	60 Bed n (%)	25 Bed n (%)	20 Bed n (%)	15 Bed n (%)	10 Bed n (%)	P Value
1.	My hospital has an infection Control team.	Yes	20(15)	27(20)	29(22)	38(28)	20(15)	0.056
		No	48(22)	42(19)	45(29)	34(16)	47(22)	
2.	The infection control Practitioner is physically present/ on call during every shift.	Yes	21 (27)	15(19)	19(24)	10(13)	12(16)	0.243
		No	47(17)	54(20)	55(20)	62(23)	55(20)	
3.	Hospital has made Improve HH adherence an institutional priority	Yes	21(17)	27(22)	31(26)	15(21)	27(22)	0.146
		No	47(31)	42(18)	43(20)	57(25)	40(17)	
4.	Hospital provides hand disinfectant in every ward and patient's room.	Yes	38(24)	23(15)	33(21)	21(13)	41(26)	0.006
		No	30(15)	46(23)	41(21)	51(26)	26(13)	
5.	Hospital provides Suitable hand disinfectant in patient 'room and in corridors.	Yes	30(20)	27(18)	37(25)	21(14)	33(22)	0.192
		No	38(19)	42(21)	37(18)	51(25)	34(17)	
6.	Hospital has no multiple use towelsfor staff use.	Yes	22(18)	23(19)	36(30)	18(15)	22(18)	0.046
		No	46(20)	46(20)	38(17)	54(24)	45(20)	
7.	My hospital provides disposable paper towels for hand disinfection	Yes	15(12)	27(27)	30(23)	38(30)	21(16)	0.004
		No	53(24)	42(19)	44(20)	34(16)	46(21)	
8.	Facility has conspicuous written material on transmission, of nosocomial infection	Yes	12(11)	20(18)	27(24)	31(27)	23(20)	0.021
		No	56(24)	49(21)	47(20)	41(17)	44(19)	
9.	Hospital provides disposable thermometer for patients in isolation.	Yes	8(12)	13(20)	14(21)	22(33)	9(14)	0.041
		No	60(21)	56(20)	60(21)	50(18)	58(20)	
10.	Patient's family members question HCW if they determine that the HCW intends to examine patients without hand washing.	Yes	19(31)	27(19)	33(23)	34(24)	31(21)	0.030
		No	49(24)	42(20)	41(18)	38(18)	36(17)	
11.	The administration provides workers with incentives to participate in educational courses on transmissionof HAIs.	Yes	8(10)	16(19)	22(27)	13(16)	23(28)	0.023
		No	60(22)	53(20)	52(19)	59(22)	44(16)	
12.	The administration in facility mandates HCW participation in c educational courses on prevention of HAIs.	Yes	7(6)	28(22)	28(22)	25(20)	39(31)	0.000
		No	61(27)	41(18)	46(21)	47(21)	28(13)	

Regarding the availability of literature for awareness of nosocomial infection for staff in health facility, n=31(27%) health workers of 15 beds hospital and n=27(24%) of 22 beds hospital HCW have responded positively. When employees ask about the availability of disposable non-critical medical device (Thermometer) for patients in isolation facility n=22(33%) workers of 15 beds hospital responded positively followed by n=14 (21%) staff members of 22 beds hospital.

Regarding patient's family question about the hand washing practice of HCW, the respondents belonging to 60 beds hospital n=19 (31%) give positive response flowed by 15 beds hospital n=34(24%).

15 beds hospital n=23(28%) and 22 beds hospital is n=22(27%) respondents affirmed that their hospitals facilitate them to participate in educational activities on infection control.

Regarding the role of administration in CME, n=39(31%) HCW of 10 beds hospital and n=22(25%) HCW of 15 beds hospital affirmed that it is mandatory to participate in CME oninfection control.

The questions which were related to hospital preparedness regarding infection control practices were converted to numeric data and all practices with a YES was given a score of 1 and NO was given 0. The scores were than computed and after fulfilling the assumptions ANOVA was applied for finding difference in preparedness among different hospitals categorized on number of beds. Significant difference was observed among the hospitals (F 2.698, P value 0.03). Table 2. When pairwise comparison was performed through application of Post hoc Tukey, significant difference was only found between 22 bedded and 60 bedded hospitals (p value 0.029).

Frequency of nosocomial infection

Hospital Acquired Infections data taken from the medical record of patients from July 2016 to Dec 2016, from 5 healthcare facilities. HAIs includes were,surgical site infections, Catheter-associated Urinary Tract Infections and Blood Stream Infection. Frequency of HAIs increases with increase in hospital bed Occupancy (Figure 1). Highest in 60 beds hospital and lowest in 10 beds hospital.

Overall infection control knowledge among HCWs was poor 23%. However, n=266 (76%) of HCW knew safety precaution for safe disposal of used medical supplies like needles, syringes, and catheters which transfer Nosocomial Infection to healthcare workers. n=230 (65%) of HCW have knowledge that hospitals harbor microorganism that could be transmitted by HCW. Only n=266 (23%) know mode of transmission of HAIs. n=172 (22%) knew that they are supervised while washing hand. Knowledge of hand hygiene after removing sterile or non-sterile gloves was only n=162 (21%) among HCW. Awareness about hand washing guideline was only n=249 (20%) among HCW. Only n=244 (18%) knew that an alcohol-based hand sanitizer was effective.

Table 2: **Difference in mean preparedness scores among the hospitals**

				95% Confidence Interval for Mean			
# of Beds	**N**	**Mean Std.**	**Deviation**	**Lower Bound**	**Upper Bound**	**F**	**P value**
10	67	4.4925	2.65360	3.8453	5.1398		
15	72	3.9722	2.39114	3.4103	4.5341		
22	74	4.5811	2.81389	3.9292	5.2330	2.698	0.031
25	69	3.9565	3.26043	3.1733	4.7398		
60	68	3.2500	2.24855	2.7057	3.7943		

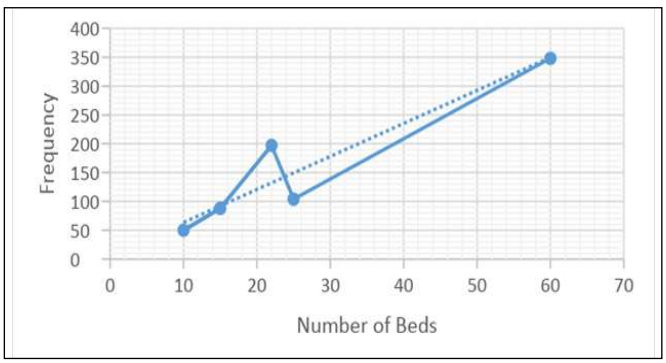

Fig 1. Frequency of nosocomial infection and hospital bed occupancy

Discussion

The purpose of this study was to determine the frequency of nosocomial infections and to assess the knowledge of health care workers (HCWs) with regard the spread of HAIs (Table 3).

Among the respondents n= 201 (57.4%) are female and n=149 (42.6%) are male. These finding are similar to those documented in the study of Sessa *et al*.[18]

Most of the participant n=123 (35.1%) lies between the age 21-30 years, this was same to study conducted by Johnson *et al*. 2013; Janjua *et al*., 2007, Reda *et al*., 2010.[19-21]

Regarding the education, n =97 (27.7) participants has only 1-year education in their respective field while only 8.9% (n=31) are Registered Nurse with 3 years diploma. Similar views were found in study conducted in Baghdad.[22]

In term of field experience of participant n=219 (62.6 %) have more than 3 years of experience in fields. This finding is similar to the study conducted by Taheri and Jokar, 2007[23] which indicate that most of sample has less than 5 years of employment in the hospital.

Majority of the respondents of study 292 (83.4 %) said that they participated in training dedicated to infection control and n=135(38.6%) of the respondents had attended educational

Table 3: Healthcare provider's perception regarding Nosocomial Infection

#	Knowledge	Response	Nurses n (%) n=128	Pharmacists n (%) n=22	Doctors n (%) n=69	Ward boys n(%) n=72	Sweepers n (%) n=59	P Value
1.	I am aware of hand washing guideline.	Yes	88 (35)	13(4)	61(27)	52(19)	35(16)	0.005
		No	40(9)	9(9)	8(6)	20(18)	24(21)	
2.	I have been supervised during hand washing activity.	Yes	70(42)	9(8)	28(17)	42(21)	23(20)	0.018
		No	58(36)	13(5)	41(20)	30(17)	36(20)	
3.	Health facility harbor microorganism that could be transmitted by HCW.	Yes	81(31)	10(7)	56(27)	48(62)	35(47)	0.000
		No	47(48)	12(7)	13(10)	24(16)	24(16)	
4.	I know how to use biohazards bags/containers.	Yes	75(34)	13(5)	46(24)	46(21)	36(14)	0.514
		No	53(43)	9(5)	23(14)	26(21)	23(16)	
5.	I know how and where the contents in biohazards bag/container are disposed.	Yes	76(35)	14(7)	42(18)	46(25)	33(14)	0.331
		No	52(37)	8(16)	27(16)	26(20)	26(19)	
6.	I know safety precaution for disposal of needles ect.	Yes	91(35)	15(19)	60(64)	58(21)	42(14)	0.066
		No	37(47)	7(7)	9(8)	14(10)	17(16)	
7.	Nosocomial infection may be transmitted via medical equipment.	Yes	95(35)	16(19)	65(64)	58(21)	17(14)	0.001
		No	33(47)	6(8)	4(5)	14(27)	42(12)	
8.	Hand hygiene should be performed after using sterile or non-sterile glove.	Yes	85(36)	12(4)	54(23)	48(25)	43(17)	0.098
		No	43(43)	10(8)	15(11)	24(17)	16(19)	
9.	I am aware of guideline for hand hygiene with alcohol based handsanitizer.	Yes	85(34)	14(5)	52(25)	53(18)	40(10)	0.158
		No	43(44)	8(7)	17(15)	19(14)	19(18)	

workshops 6 months to 1 year ago. Among 350 health professionals only 57 (16.3%) had ever participated in any training program about infection prevention. Angelillo *et al*.[24] recommended that educational courses about HAIs had a positive effect on infection control procedures and compliance.

Most of the respondents 62.3% of this study showed that management of healthcare facilities did not arrange continuing education courses on hospital infections control. Angelillo *et al*.[24] study demonstrated that continuous education courses on HAIs control had improve health care providers'adherence to infection control measures. But in this study however 87.7% of participants have attended educational workshops on infection control but overall knowledge on infection control was poor. This may be due to poor presentation or language barriers. This needs further research on effectiveness of educational workshops on infection control measures. In present study, total score for knowledge was 23%. Many studies have reported level of knowledge regarding infection control in health care providers and it ranges 16% to 75%. A research conducted in Nepal showed 16% of HCWs had such knowledge.[25] While Study in Jordon reported that 46% of HCW had infection control knowledge.[26]

In this study n=212(60,6%) of respondent showed that their facility has no Infection Control Committee. The Infection

Control Committee is an important part of infection control program as committee supervises infection control practice in hospital. It also recommends and implements appropriate policies, and frequently reviews these policies.[27]

The awareness to hand washing guideline was evaluated by asking leading question like when should you wash hands and how should you wash your hand? Knowledge about the steps of hand washing. What should you do if you don't have soap and clean water? Out of 350 respondents, 228 (65.1%) of HCWs thinks that promotion of hand hygiene adherence is not their hospital priority. Hand hygiene practices rate remain low in hospitals generally despite the documented guidelines. An average adherence rate is 40% as it reported in 34 studies from 1981 to 2000.[28]

n=194 (55.4%) of the respondents stated that administration not provide hand hygiene disinfectant in facility. WHO recommends alcohol-based hand formulations as disinfectant, at the point of care. Dispensers' location at the point of care makes hand hygiene easier by overcoming barriers such as inconvenient dispenser locations or lack of disinfectant agents.[29,30]

In this study only n=88(35%) nurses and n=61(27 %) doctors 19% of janitorial staff and 16% of sanitary staff were aware guideline of hand hygiene. Hand washing is key to the prevention of spread of micro-organisms responsible for nosocomial infections, but frequently, this is not adequately recognized by HCWs.[3,32]

In this study 35% of nurses and 27% of doctors have knowledge about hand washing. This is in contrast to study conducted by Manishaat *at al*.[33] where doctors' knowledge was 92.9% while nurses' knowledge was 88.4%.

In this study only 19.6% of HCW responded said that they had a sharp disposal system in various working stations. This needed to be improved by hospital quality improvement program, HCWs education and regular audits.[34]

In this study only 35% of nurse, 21% of supporting staff and only 14% of sanitary staff has knowledge of safe disposal of used syringes/needles and other sharps. As shown in this research nurses had relatively better knowledge of infection control as compare to other HCWs like technician ect. Study by Taneja *et al*. reported that 75.5% of staff nurses at a tertiary care hospital had knowledge of infection control.[35]

n=162 (21%) of respondents used to wash hand after removing sterile /non-sterile gloves. Gloves may be torn during use or may have small, unapparent defects, so during removal of gloves hands can become contaminated.[36] Hand hygiene is thus essential before changing another pair of gloves. In present study only 34 % of nurses aware of guideline for hand hygiene with alcohol-based formulation. Alcohol-based disinfectants found to have superior activity in reducing bacterial counts in hospitals.[37,38]

This study showed that frequency of HAIs increased with increase of hospital occupancy. Increase nursing workload is one of the main risk factor for HAIs.[39,40] If the nursing staff is not proportionally increase with high bed occupancy, HAIs increases. The highest prevalence of HAI was reportedin large and teaching hospitals (6.7% and 7.4%, respectively).[41] Studies have shown that overcrowding and understaffing leads to failure of patient safety programs as it decreases healthcare worker adherence to hand hygiene and overburden the screening and isolation facilities.[42]

Conclusion and Recommendation

The result of this research showed that the present state of healthcare provider's knowledge related to Nosocomial infection was not satisfactory. Policies on infection control and training andre-training of HCW are highly recommended. Hospital administration should provide resources and support in the form of educationand training opportunities to health care personals. There is urgent need of Infection Control guidelines in every hospital for HCWs. It is also recommend displaying written guidelines in every health care facility. Monitoring system for infection rates is needed in every hospital. There is urgent need of Infection Control Committee in each institution. Continuous training program of health care providers, implementation of policies about infection control and adherence to practicesof infection control are effective in control of HAIs. Further study required to compare effect of knowledge of HCWs on infection control practices.

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ORIGINAL ARTICLE

Surveillance of Inducible Clindamycin Resistance of *Staphylococcus aureus* in Hyderabad.

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Abstract

Objective
To evaluate and analyze the inducible and constitutive clindamycin resistance of *Staphylococcus aureus* from various clinical samples (i.e. blood, wound, nose, and urine in Hyderabad.

Methodology
Staphylococcus aureus isolates from various clinical specimens were included in this study. The sensitivity profiling against test antibiotics and determination of Inducible clindamycin resistance was performed by traditional Kirbeybaur disc diffusion and D-test respectively.

Results
The percentage of erythromycin resistant strains was 37% (n=56), of these ~43% (n=24) displayed constitutive clindamycin resistance while ~57% (n=32) were sensitive to clindamycin. Approximately two-third, 63% (n=20) of Clindamycin-Erythromycin-Discordant strains displayed $iMLS_B$ phenotype. The percentage of $iMLS_B$ phenotype in *Staphylococcus aureus* recovered from male and female was 64% (n=16) and 57.1% (n=4) respectively. OR was 1.33 [CI (95%) = 0.24-7.34]. Statistically non-significant differences (p-value >0.05) for $iMLS_B$ phenotype between genders were seen. $iMLS_B$ phenotype was maximum in case of blood isolates (87.5%) followed by wound isolates (70%). The highest percentage of $iMLS_B$ phenotype was seen in the age group of 51-60 years. Pearson's co-relation co-efficient and p-value were determined to be ($r=0.639$, $p<0.05$), suggesting a significantly positive co-relation between various age groups and $iMLS_B$ phenotype.

Conclusion
The $iMLS_B$ phenotype of *Staphylococcus aureus* is independent of gender and significantly positively co-related with increasing age of the patients.

Key words
S. aureus, Clinical specimen, $iMLS_B$, $cMLS_B$, Clindamycin, Hyderabad.

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Introduction
The growing interest in the resistance of *Staphylococcus aureus* (*S. aureus*) to various antibiotics in the last few decades[1] has led to the use of alternative agents such as macrolide, lincosamide and streptogramin B (MLS) family that act through the inhibition of protein synthesis.[2] Clindamycin is an efficient and economic lincosamide drug used for the treatment of staphylococci infection.[3] Clindamycin has several advantages in the treatment of *S. aureus* infections. It can be administered intravenously and orally with good bioavailability. It penetrates the skin and soft tissue easily, exerts an inhibitory action on toxin production, and is relatively inexpensive. Furthermore it is a useful choice in case of penicillin allergy.[4] In *S. aureus* the resistance against clindamycin can occur through the enzymatic inactivation of lincosamides antibiotic. Such a resistance is mediated by the *inuA* gene, however this is rare.[5] The *S. aureus* also develops resistance against clindamycin through methylation of target site, which is generally due to the cross-resistance developed against erythromycin.[6,7] Soon after the introduction of erythromycin (a Macrolide) into therapy, in 1956, resistance against it was witnessed in staphylococci.[8] Among the two types of resistance mechanisms documented for Erythromycin in *S. aureus*, the enzymatic modification of target site is common.[9] The resistance due to methylation of the target site (i.e. 23S ribosomal RNA) also leads to cross-resistance to other macrolides, lincosamides, and streptogramins B, and is named as MLS_B phenotype.[10] The MLS_B phenotype is encoded by *erm* (erythromycin ribosome methylase) gene,[11] and a variety of *erm* genes have now been reported in a large number of microorganisms.[5]

Studies have also divided the MLS_B phenotype in two categories i.e. $cMLS_B$ (constitutive) and $iMLS_B$ (inducible).[12] The $cMLS_B$ renders the strains stably resistant to macrolides, lincosamides, and streptogramins B while, the $iMLS_B$ is induced by low level of erythromycin.[8] The $iMLS_B$ in *S. aureus* can severely compromise therapy and can result in failure of clindamycin treatment of *S. aureus* infections when non-suitable therapy (e.g. erythromycin) is given.[13] The $iMLS_B$ can complicate therapy when $iMLS_B$ phenotype possibly switches into $cMLS_B$.[9] A D-test can differentiate between $cMLS_B$ and $iMLS_B$ in erythromycin resistant strains. A positive D-test suggests the presence of an *erm* gene that could result in constitutive clindamycin resistance and clinical failure.[9]

Although a great number of studies have consistently investigated the occurrence and prevalence of iMLSB among *S. aureus*.[4,14,15] The prevalence varies by bacterial susceptibility profile, bacterial species, geographic location, and patient age.[16] The data pertaining to Hyderabad regions have never been collected. We therefore wish to investigate the same to ultimately add in the current strategies for empirical therapy against *S. aureus* infections in Hyderabad regions.

Methodology

Chemicals and Media

All the media i.e. Mannitol Salt Agar, Muller Hington Agar and Nutrient broth were purchased from Oxide. Erythromycin and clindamycin impregnated discs (5µg and 2µg, respectively) were from oxide.

Staph aureus isolates

The S. *aureus* isolates used during this study were obtained from Civil Hospital Hyderabad, Diagnostic and Research Laboratory, LUMHS and a few private diagnostic laboratories in Hyderabad. All these isolates were of clinical origin specifically isolated from clinical specimens i.e. blood, wound, nose, and urine. From January 2015 to January 2016 a total of 150 identified *S. aureus* isolates were collected. The study was approved by Board of Advanced Studies and Research [No.DRGS/426] University of Sindh, Jamshoro. Following parameters were used to determine the minimum sample size (n = 87) for $iMLS_B$ phenotype in *S. aureus*; the Z value of standard normal distribution was calculated from 95% (CI) with a margin of error of 5%, and about 6% of previously reported $iMLS_B$.[17]

Antibiotic sensitivity test

The antibiotic sensitivity testing against erythromycin and clindamycin was performed using *Kirby-Bauer* Disc Diffusion method. The liquid cultures of *S. aureus* were prepared in nutrient broth. The overnight culture was diluted to OD_{600} = 0.5. to meet the McFarland's standard required for disc diffusion method. Sterile cotton swab were used to inoculate the diluted culture on Muller Hington Agar and spread evenly. The antibiotic discs (*Oxoid*) were placed on the agar surface. In order to get a flat contact with agar surface a gentle pressure was applied on the discs. The plates were incubated at 37°C for 24 hours. The diameter of the clear zones (zones of inhibition) observed around the antibiotic discs were measured according to Clinical and Laboratory Standard Institute (CLSI).

D-Test for the determination inducible clindamycin resistance.

The $iMLS_B$ phenotype was detected by performing a D-test according to CLSI guideline. Briefly, OD_{600nm}= 0.5McFarland equivalent suspension of organisms was inoculated onto a Mueller Hington agar (MHA) plate, both erythromycin and clindamycin discs were placed 15-26 mm (edge to edge) apart on agar surface. The plates were then incubated at 37°C for about 24 hours. A D-shaped zone formed due to the induction of resistance by erythromycin in clindamycin sensitive strain was recorded as positive D-test.

Statistical Analysis

The data analysis was done using Microsoft excel and online statistic software. The individual as well as collective levels of resistance against erythromycin and clindamycin in *S. aureus* were measured both in terms of absolute and relative values. The percentage of strains expressing $iMLS_B$ phenotype and their relevance to gender and various clinical samples were the variable of interest. The Odds Ratios (OR) and CI with 95% were calculated manually and using a statistic calculator. The *p*-values were calculated using a Fisher's Exact test of independence.

Results

A total of 150 identified *S. aureus* isolates recovered from blood, urine, wound and nose, were included in the current study. One hundred and nine (109) of these were from male patients while 41 were from female patients. Maximum number (n=68) isolates were from nose followed by urine (n=36). All the strains were processed for antimicrobial sensitivity against erythromycin and clindamycin by traditional Kirby baur disc diffusion test. The overall percentage of resistance against erythromycin was found to be 37% (n=56), of these 42.8% (n=24) displayed constitutive clindamycin resistance while 57.2% (n=32) were sensitive to clindamycin (Table 1). These erythromycin-clindamycin (EC) discordant strains were then subjected to a D-Test for the detection of $iMLS_B$ phenotype. The $iMLS_B$ phenotype was detected in 62.5% (n=20) of the EC discordant strains. Categorically higher percentage of $iMLS_B$ phenotype was seen in case of isolates from male patients (64%) than female patients (57.1%) (Figure 1). To evaluate the differences the OR = 1.33 with CI (95%)= [0.24-7.34] was calculated. The analysis using Fisher's Exact test of independence suggested a statistically non-significant level of difference (p=1.000, α=0.05).

Among various clinical specimens, the maximum display of $iMLS_B$ phenotype was witnessed in case of blood isolates (88%) while the minimum was seen in case of nose isolates (20%). The $cMLS_B$ phenotype was most common in case of nose isolates (62%). Figure 2 displays the bar diagram for comparative percentages of $iMLS_B$ and $cMLS_B$ phenotypes among the isolates of various clinical origins.

We also analyzed the data for the display of $iMLS_B$ and $cMLS_B$ phenotype with reference to the age of the patients. The data were categorized in various randomly selected age groups. The magnitude of the age was selected to be 10. Figure 3 displays the percentages of $iMLS_B$ and $cMLS_B$ phenotypes in various age groups. The maximum percentage of $iMLS_B$ and $cMLS_B$ phenotypes were seen in case of age group 51-60 years i.e. (100% and 66.6%, respectively). To determine the co-relation between age of the patient and $iMLS_B$ phenotype (if any) we

performed bivariate co-relation analysis. The Pearson's co-relation co-efficient and p-value were determined to be (r=0.639, *p*=0.00001), suggesting a significantly positive co-relation between various age groups and $iMLS_B$ phenotype.

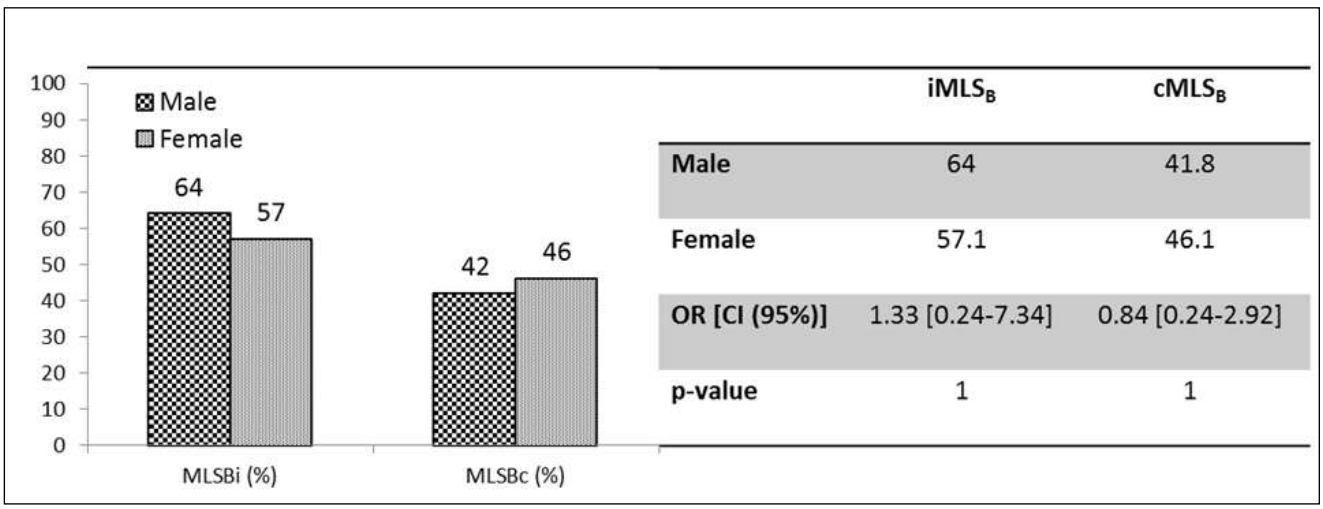
Discussion

Clindamycin is an efficient and economic lincosamide drug used for the treatment of staphylococci infection.[3] Though structurally different the Clindamycin functions similar to Macrolide, other lincosamides and Streptogramin family of antibiotics. All these affect the *S. aureus* growth by interfering the protein synthesis through their binding to the 23S rRNA on the 50S subunit of the bacterial ribosome.[7] The resistance against clindamycin can develop either due to *inuA* gene or can

Table 1: Table showing the absolute and relative values (expressed in percentage) for $iMLS_B$ and $cMLS_B$ phenotypes.

Specimen	*S. aureus* (n)	Ery [R] (n)	Clin [R] (n)	Ery [R]-Cli [R] (n)	EC-Discordant (n)	D - Positive (n)	D - Negative (n)	$iMLS_B$ %	$cMLS_B$ %
Nose	68	21	14	13	8	3	5	37.5	61.9
Wound	27	16	9	6	10	7	3	70	37.5
Blood	19	10	3	2	8	7	1	87.5	20
Urine	36	9	4	3	6	3	3	50	33.3
Total	**150**	**56**	**30**	**24**	**32**	**20**	**12**	**62.5**	**42.8**
Gender									
Male	109	43	22	18	25	16	9	64	41.8
Female	41	13	8	6	7	4	3	57.1	46.1
Total	**150**	**56**	**30**	**24**	**32**	**20**	**12**	**62.5**	**42.8**
Age-groups									
01 -to- 10	1	0	0	0	0	0	0	0	0
11 -to- 20	35	14	8	6	8	5	3	62.5	42.8
21 -to- 30	50	18	9	9	9	6	3	66.6	50
31 -to- 40	40	12	5	4	8	4	4	50	33.3
41 -to- 50	17	9	6	3	6	4	2	66.6	33.3
51 -to- 60	7	3	2	2	1	1	0	100	66.6
Total	**150**	**56**	**30**	**24**	**32**	**20**	**12**	**62.5**	**42.8**

R = Resistant, EC = Erythromycin Clindamycin, Ery = Erythromycin, Clin = Clindamycin.



	$iMLS_B$	$cMLS_B$
Male	64	41.8
Female	57.1	46.1
OR [CI (95%)]	1.33 [0.24-7.34]	0.84 [0.24-2.92]
p-value	1	1

Fig 1. (Left) A bar diagram describing the percentages of $iMLS_B$ and $cMLS_B$ phenotype in *S. aureus* strains isolated from both male and female patients. (Right) Table describing the OR and p-values

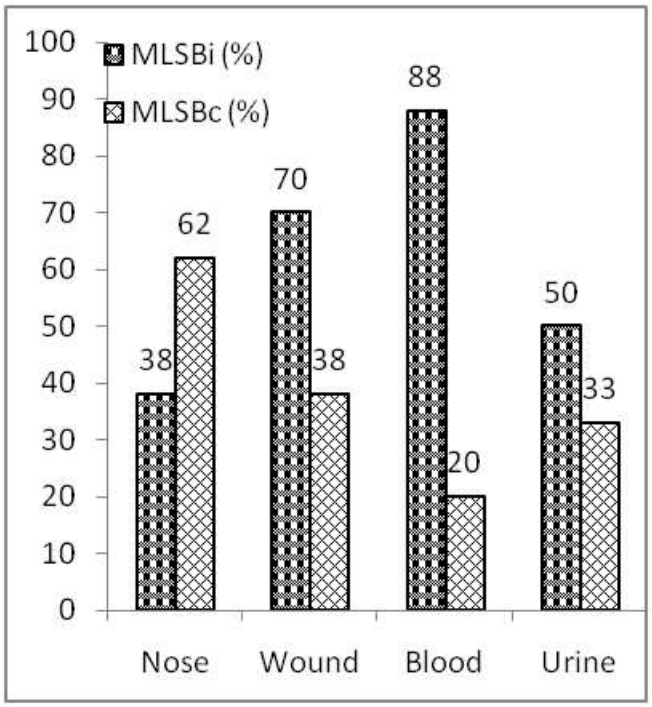


Fig 2. A bar diagram describing the percentages of iMLS$_B$ and cMLS$_B$ phenotype in *S. aureus* strains isolated from various clinical samples.

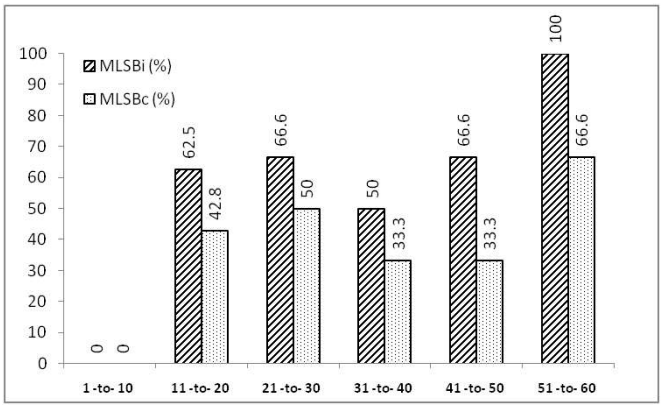


Fig 3. A bar diagram describing the percentages of iMLS$_B$ and cMLS$_B$ phenotype in *S. aureus* strains isolated from patients of various age groups.

be induced due to erythromycin ribosome methylase encoded by *ermA* gene in *S. aureus*. The current study was performed to evaluate the frequency of inducible type of clindamycin resistance in clinical *S. aureus* isolates. Out of 150 *S. aureus* isolates from various clinical specimens 37% (n=56) were resistant to erythromycin. The constitutive clindamycin resistance was seen in 16% of the total *S. aureus* included in this study. A study conducted in 2016 (Israel) reported about 26.8% of the constitutive Clindamycin-resistance.[8] Previously Fasih N. and others.[14] reported about 30% of the constitutive clindamycin resistance in Pakistan. In the current study, we extended our analysis to more specific outcomes. We calculated the percentages of inducible and constitutive types of clindamycin resistance in 56 Erythromycin resistant isolates. Twenty four (42.8%) of these isolates displayed the cMLS$_B$ phenotype while thirty two (57.2%) were sensitive to clindamycin. The potential inducer of inducible clindamycin resistance in *S. aureus* is erythromycin in erythromycin resistant strains. We therefore determined the inducible clindamycin resistance in these strains using a phenotypic D-Test. Out of these 32 EC-discordant strains the iMLS$_B$ was seen in 62% (n=20) of the isolates. Six (6%) and seventy two percent (72%) of the iMLSB has been reported based on a two independent studies conducted on clinical *S. aureus* isolates from Karachi.[14] and Lahore[17]. We calculated the OR and CI(95%) to determine if the iMLS$_B$ is affected by gender or not. The statistical analysis suggested non-significant differences of iMLS$_B$ phenotypes between genders. The data was also analyzed to determine the relationship (if any) between iMLS$_B$ phenotypes and the age of the patients. Various age groups with uniform class magnitude were formed. The frequency of D-positive cases in each group was expressed in relative values. The Pearson's co-relation co-efficient and *p*-value were determined, the results suggested a significantly positive co-relation between various age groups and iMLS$_B$ phenotype. Given the usefulness of clindamycin antibiotic in the treatment of *S. aureus* infections and the scarcity of relevant published literature based on the data in Pakistan, the current findings will play valuable part in the management and rational formulation of antibiotic regime.

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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.

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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.

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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**.

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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.

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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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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.

Instructions updated - April 2012.
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