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Clinical Characteristics and Risk Factors of Candidemia in Tertiary Care Hospital

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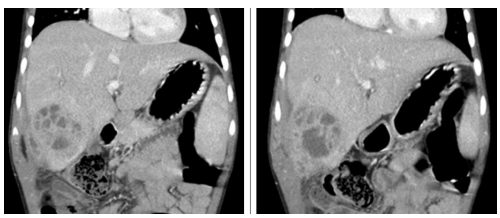
CONTENTS **PAGE #**



3 ½ year old boy with multicystic area in liver extending upto skin. His Echinococcocus titer became positive.
Diagnosis Hydatid Cyst

Courtesy: Dr Ali Faisal Saleem, Aga Khan University, Karachi.

Combating Childhood Pneumonia and Diarrhea at Community Level: Potential of m Health to improve interaction among health workers

Pneumonia and diarrhea are the leading causes of mortality and morbidity among children under five. Together, these two early childhood infections lead to one-fifth of under-five mortality across the globe.[1,2] Major proportion of these deaths occur in the resource constrained settings of developing and underdeveloped world.[3]

In Pakistan, approximately 91,000 child deaths are attributable to pneumonia and 53,000 to diarrhea annually thus contributing to 46% of overall under-five mortality. Due to suboptimal performance of country's health system the target of improving child survival by bringing down deaths from pneumonia to fewer than three children per 1,000 live births and from diarrhea to less than one in 1,000 remains a labor intensive challenge.[3]

Pakistan has a three tiered public health system consisting of primary, secondary and tertiary health care facilities. Covering 60% of rural population and working through 130,000 Lady Health Workers (LHWs), the Lady Health Worker's Program (LHW-P) represents the outreach community based component of this system. It is meant to work in coordination with the primary care facilities: basic health units (BHUs) and rural health centers (RHCs). Lady Health Supervisors (LHSs) are responsible for on-going supervision and mentoring of LHWs. Community Case Management (CCM) for childhood diarrhea and pneumonia constitutes an important component of the LHW-P. Ironically, LHWs are not well integrated into the broader public health sector and cases referred by LHWs receive little priority at facility level. It is therefore not surprising that there have been negligible improvements in the coverage of essential interventions. According to an estimate, only 38% cases with diarrhea received ORS and antibiotics were administered to 41% pneumonia patients.[4]

The fourth evaluation of the LHW-P has indicated that weak supervision of LHWs by LHS is an important determinant of the stagnant under five mortality.[5] One of the important reasons identified for this feeble supervision is the lack of coordination and interaction between LHWs and their LHSs. Insufficient interaction among health workers is evidenced by the fact that 40% of the LHSs do not conduct the required household supervisory visits, only 28% LHWs were informed about their performance scores in writing during a supervisory meeting at the facility while 61% LHWs did not receive any type of feedback (verbal or written) from their supervisors. Lack of transport, refresher training,regular salary and accessibility to the assigned areas were some of the barriers preventing LHSs to serve as effective supervisors.

Project NIGRAAN (2013-2015), a cluster randomized implementation research project addressed this coordination and communication gap between LHWs and LHSs in the presence of existing logistic barriers. NIGRAAN used simple cell phones (<30 US $ per unit cost) for enhancing timely case reporting by LHWs and supervisory follow up visits by LHSs. Short Message Service (SMS) was utilized to track cases of childhood diarrhea and pneumonia within the existing Management Information System of LHW-P in District Badin, Sind. 34 LHSs and 170 LHWs were enrolled. LHSs were given cell phones for real-time communication with LHWs while LHWs were provided a minimal monthly communication allowance to purchase SIM cards. LHWs upon case identification relayed information and arranged appropriate follow up visits with LHSs via mobile. Total 6128 cases were tracked (diarrhea: 3058; pneumonia: 3070). Between the first and final quarter of surveillance, LHW to LHS case reporting via SMS improved from 43% to 98%. Almost all cases were reported by LHWs to LHSs within 24 hours of identification. LHSs followed up more than 60% of reported cases at the household and also provided LHWs written feedback.The specific outcome of the illness was tracked via cell phones 72 hours later; 47% cases were found to be recovering with private treatment, 26% with government facility treatment and 3% with LHW treatment. ORS was provided to 23% diarrhea and antibiotics to 2% pneumonia cases by LHWs. This may be attributable to shortage of supplies with LHW-P. Referral rates to other facilities were high especially for cases with pneumonia.

According to a report, mobile technology has already contributed to promote health care services in India, Bangladesh and Botswana.[6] Mobile health (m health) based interventions enhance efficiency of service delivery ensuring timely feedback and supervision by the health managers through effective communication. The same report shows that in Uganda and Rwanda, m Health improved child health by facilitating remote diagnosis and treatment. In Pakistan, NIGRAAN is one of the first studies to demonstrate the possibility of obtaining child health related data from marginalized rural areas in real time using a m health intervention. It has shown that m Health is technically feasible, usable and acceptable by frontline health workers.The study has also demonstrated that using m health LHW-P has the potential to enhance interaction and coordination between LHWs and LHS, identify cases, report, manage and refer them to the next level of care.

With this growing body of evidence it is time to invest in wide scale deployment of mobile technology in developing countries where access to good quality health care remains an unachievable goal for the poor and marginalized. The LHW Programme in Pakistan provides readily available health services at community

door steps and thus offers a very good investment opportunity to strengthen the fragile health system of Pakistan using m health.

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ORIGINAL ARTICLE

Underlying Etiology of Cervical Lymphadenopathy in Children in a Country Endemic for Tuberculosis

Sohail Asghar Dogar,* Ahmad Vaqas Faruque,* Muhammad Arif Mateen Khan*, Ali Faisal Saleem**

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Abstract

Paediatric cervical lymphadenopathy (CLAP) is a common presentation in pediatric practice. A biopsy is indicated if it fails to regress in size within 4-6 weeks after appropriate medical therapy. We aim to report the frequency of the various causes of lymphadenopathy in children as determined by histopathology.

Methods
This is a retrospective review of 170 children who underwent excisional biopsy of the cervical lymph node from 1988-2011. Data was extracted from the health information management system. Data was collected on gender, histopathological findings, and age at presentation. Bivariate analysis (by using chi-square test) was used to identify any significant differences between outcome categories (benign or malignant) with respect to the independent variables. P-value and 95% confidence intervals were calculated and p-values < 0.05 were considered significant.

Results
Persistent low grade fever with or without weight loss was the commonest indication for biopsy. Histopathology showed benign disease among 77% (n=131). Amongst the benign causes, tuberculosis (n=55, 42%) was the most common while lymphoma (n=32, 19%) was the most common malignant cause of CLAP. Children older than 5 years of age were 1.6 times more likely to have a malignant lesion with persistent CLAP unresponsive to antibacterial therapy (p=0.17, 95% CI, 0.7–3.7). Malignant lesions were 7.1 times more common time males (p=<0.001, 95% CI, 2.6–19.3) and 3.2 times more with multiple enlarged CLAP (P=0.01, 95% CI, 1.2–8.8).

Conclusion
Benign diseases (reactive lymphadenopathy and tuberculosis) remain the most common cause of persistent cervical lymphadenopathy. However male gender, age> than 5 years and multiple cervical lymph nodes should alert the physician towards evaluation for more sinister diagnoses.

Key words
Persistent Cervical Lymphadenopathy, Paediatrics, Tuberculosis, Lymphoma

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Introduction
Paediatrics Cervical lymphadenopathy (CLAP) is one of the most common and challenging presenting findings in outpatient pediatrics. Most of them are bengining with a reactive process (infectious or inflammatory) that usually self-resolving without any sequels. Reactive hyperplasia is mostly because of bacterial, viral or protozoal infections but malignancies are always in the list of differential diagnoses.[1,2] Up to 40-60% of paediatrics population has CLAP at some point in their life.[3,4] Cervical lymph nodes drain the head and neck region of the body. A thorough history and physical examination is the key in understanding the disease process and its likely differential diagnoses.[5] A biopsy is indicated if CLAP doesn't respond to conventional antibacterials. The purpose of this study is to determine the common etiologies identified after excisional cervical lymph node biopsy in children that did not respond to first line conventional antibacterial in tuberculosis endemic setting.

Methods
We performed an audit of all cases of pediatric CLAP who underwent excisional biopsy in the last 23 years (1988 to 2011) at the Aga Khan University hospital, Karachi, Pakistan. Prior approval was taken from our medical record audit committee. Aga Khan University is a major tertiary care hospital in the middle of the metropolitan city of Karachi. We included all children who were aged 15 years or younger, and presented with enlarged cervical lymph nodes which failed to regress after appropriate antibacterial therapy, and were then referred for an excisional biopsy to the pediatric surgeon during the mentioned period. Cases were identified via two sources; i) Hospital information management system (HIMS) by using international classification of disease (ICD-9-CM) codes: – a) Diseases; 785.6 (enlarged lymph nodes) b) Procedures; 40.21 (excision of deep cervical lymph nodes); 40.3 (regional lymph node excision); 40.4 (radical excision of cervical lymph nodes), and ii) hospital internal histopathology registry to confirm that the procedure was performed in-hospital . Children with excisional biopsies performed outside AKU hospital were excluded. Histopathological findings (benign or malignant) was the outcome variable, while gender, age at presentations, presenting complaint, single or multiple lymph node involvement, bacterial cultures etc., were the independent variables of the study. Data was analyzed by using statistical package of social sciences (SPSS version 19.0) software. Descriptive data analyses were done and the results are presented

as means and standard deviation for continuous variables (i.e., age) and proportions for categorical variables (i.e., gender, benign or malignant). Bivariate analysis (by using chi-square test) was used to identify any significant differences between outcome categories (benign or malignant) with respect to the independent variables. P-value and 95% confidence intervals were calculated and p-values < 0.05 were considered significant. A total of 170 children with complete records were available for the analysis. All the possible efforts were made to maintain the confidentiality of patients.

Results

Out of 170 children, majority were male (n=98, 58%)**.** Two third (n=112, 66%) were older than 5 years of age. Most of the children had multiple cervical region lymphadenopathy (n=122, 72%). Presenting complaints at the time of admission were fever (n=165, 97%), enlarged cervical lymph nodes (n=129, 76%) and/or generalized lymphadenopathy (n=75, 44%). Other presenting complaints includes undocumented weight loss and discharging sinus . Histopathological diagnoses was consistent with a non-specific inflammation in most of the cases (n=71, 42%). Overall histopathological finding were consistent with benign diseases (n=131, 77%). Tuberculosis (n=55, 42%) was the most common diagnosis on histopathology. Lymphoma was second most common diagnosis (n=32, 19%). Less common diagnoses included leukemia (n=5, 3%), histiocytosis, toxoplasmosis, metastatic disease, Sjögren's syndrome and Rosai-Dorfman disease (Figure 1). Cultures were sent in only 61 (36%), where majority of them did not show any growth (n=46, 75%). *Streptococcus species (n=7), Staphylococcus aureus* (n=4) and *Mycobacterium tuberculosis* (n=4) were the few organisms identified.

We divided our study subjects into two groups; the malignant, that included lymphomas, leukemia's and metastatic nodes and the benign group that included all other diagnosis. Children older than 5 years of age were 1.6 times more likely to have malignant lesions (p=0.17, 95% CI, 0.7–3.7). Malignancy was 7.1 times more common among males (p=<0.001, 95% CI, 2.6–19.3) and 3.2 times more likely with multiple enlarged CLAP (P=0.01, 95% CI, 1.2–8.8). (Table 1).

Discussion

Cervical lymphadenopathy is common in paediatric population.[2] We found an that benign lesions, non-specific reactive lymph node and tuberculosis were the commonest causes of CLAP. However a small proportion of children were diagnosed with lymphoma. Male gender, age > 5 years of age and multiple cervical lymph nodes increased the likelihood of malignancy.

Most cases of cervical lymphadenitis in children are self-limiting and can safely be monitored for spontaneous resolution over four to six weeks.[2] In 40% to 80% of cases acute unilateral lymphadenopathy is most commonly caused by streptococcal or staphylococcal infections, while acute bilateral cervical lymphadenopathy caused by upper respiratory tract viral infections. Supraclavicular or posterior cervical lymphadenopathy carries a much higher risk for malignancies than anterior cervical lymphadenopathy.

Incidence of palpable lymph nodes is 90% in children aged 4-8 years.[4] According to Larsson *et al*. approximately 38-45% of otherwise healthy children have palpable lymph nodes.[6] According to another survey, 44% of the children in a community will have palpable nodes and about 64% of the sick children in hospitals will have palpable lymph nodes. 68% of lymphadenopathies are benign in children. More than 25% of the malignant tumors in children occur in the head and neck region and the cervical lymph nodes are the most common sites. In particular, in children with chronic cervical

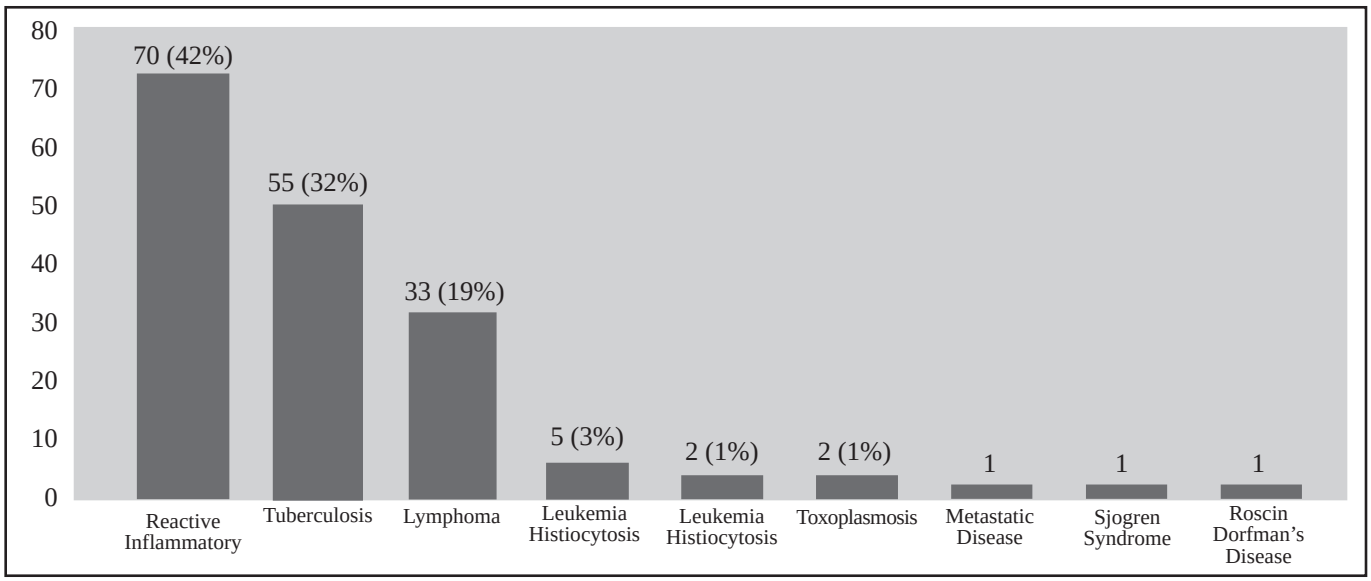

Fig 1. Spectrum of histopathological findings in the study population

Table 1. Comparison of Study participants on the basis of pathology of the disease

	Benign (n = 131)	Malignancy (n = 39)	P value	OR (95% CI)
Age of a child				
>5 years	83 (63%)	29 (75%)	0.17	1.6 (0.7 – 3.7)
Gender				
Male	64 (49%)	34 (87%)	<0.001	7.1 (2.6 – 19.3)
Pattern of cervical lymph nodes				
Multiple	89 (67%)	34 (87%)	0.01	3.2 (1.2 – 8.8)

lymphadenopathy, it is of paramount importance to exclude a neoplasm. Enlarged supraclavicular lymph nodes may be the first sign of intra-abdominal malignancy. Failure of regression after appropriate antibiotic therapy within 4-6 weeks is an indication for diagnostic excisional biopsy.[1] If there is any suspicion of malignancy or in cases of persistent lymphadenopathy an excisional biopsy with microscopic examination of the lymph node should be performed.[7,8] Excisional biopsy is still the gold standard, because in children fine-needle biopsy is difficult to perform and can be accepted as accurate in positive findings only.[9] The biopsy should be done on the largest and the firmest node that is palpable, and the node should be removed intact with the capsule.[7,8,10] Such a method is most useful in the diagnosis of lymphoma. If there is a packet of nodes, extirpation of at least 2–3 neighboring lymph nodes is necessary for the suitable histological diagnosis.[5]

When comparing these results with the international literature, we found a higher rate of tuberculosis (32%) and malignancy (19%) in our study population as compared to the other studies.[11,12] It may be because of the fact that tuberculosis is very prevalent in our society because of third world country, which is not the fact in most western countries. We have also looked at the incidence of malignancy as compared to number of lymph node. Literature is quite supportive of increase incidence of malignancy with multiple lymphadenopathies.[13] Histopathological and microbiological diagnosis is imperative prior to start any treatment.

There are few limitations, this was a retrospective study, low rate of specimens sent to microbiology and low bacterial cultures yield. It is single center study which needs further attention. The association with males could be because of the differential health seeking behaviour for male children and the high frequency of malignancy seen could be because of selective pattern of referrals for children with malignancies. This may have an impact on our results as we have found higher incidence of malignancy in children age 5 years.

Conclusion

Fever with cervical lymphadenopathy is the most common presentation. Although non-specific inflammation is the most common diagnosis but tuberculosis and malignancy are high in children in our study. Trend of malignancy is seen more in multiple enlarged cervical lymph nodes in older male children.

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Conflict of Interest

None

Funding

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ORIGINAL ARTICLE

In-vitro Evaluation of Penicillin and Ceftriaxone resistance amongst *Streptococcus pneumoniae* isolates causing meningitis, a cross sectional study at The Aga Khan University Hospital Clinical Laboratory, Karachi, Pakistan

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Abstract

Introduction
Pneumococcal meningitis is a leading cause of morbidity and mortality worldwide. Rising penicillin and ceftriaxone resistance in different regions of world has led to the guideline recommendation of adding vancomycin to ceftriaxone for empirical treatment of pneumococcal meningitis. This study aimed to determine penicillin and ceftriaxone resistance rates in *Streptococcus pneumoniae* isolates causing meningitis in Pakistan.

Methods
A prospective cross-sectional study was conducted from January 2011 to March 2014 at the Clinical Microbiology Laboratory of Aga Khan University. Forty-six pneumococcal strains from CSF and blood cultures were considered as meningeal strains according to clinical presentation and included in this study while duplicates were excluded. *S. pneumoniae* isolates were identified on colony morphology and optochin sensitivity. Minimum inhibitory concentrationof penicillin and ceftriaxone were performed by E-test method and interpreted according to CLSI.

Result
Out of 46 isolates 33% (n=15) were from patients younger than 5 years, 48%(n=22) between 5-65years while 19%(n=09) older than 65 years. Twelve strains (26.09%) were found to be resistant to penicillin; ceftriaxone and vancomycin resistance was not seen.

Conclusions
This study showed penicillin resistance to be high among the *S. pneumoniae* causing meningitis, justifying the empiric use of ceftriaxone. However, resistance to ceftriaxone was not seen, suggesting that the addition of vancomycin in empiric therapy is not required in cases that acquire meningitis locally and have no history of travel.

Key Words
Meningitis, *Streptococcus pneumoniae*, penicillin resistance, ceftriaxone, vancomycin.

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Introduction

Pneumococcal disease is a major threat to global health. It is a leading cause of severe infections such as pneumonia and meningitis.[1] According to World Health Organization, it is estimated that 1.6 million people die every year from it and majority of them are children especially in developing countries.[1] The burden of pneumococcal meningitis is largely under-investigated in Pakistan.[2] Pneumococcal Global Burden of Disease Study Team concluded that Pakistan, including six other countries of Asia, has the highest number and proportion of *Streptococcus pneumoniae* cases.[3]

Globally, there is a changing trend in the antibiotic susceptibility pattern of *S.pneumoniae* responsible for invasive infections.[4, 5] There is an increase in rate of resistance to various classes of antibiotics especially β-lactams, which have traditionally been an effective treatment.[4] This makes the treatment more difficult and costly. Resistance rates vary widely among different geographic regions. Guideline for pneumococcal meningitis recommends ceftriaxone and vancomycin as an empirical treatment.[6] Vancomycin is an expensive as well as toxic drug. Its overuse may lead to resistance not only in S*treptococcus pneumoniae* but also other commensal *Enterococcus species* and *Staphylococcus species* etc. Moreover, availability of this drug is limited in Pakistan. Resistance to penicillin, ceftriaxone or any other 3rd generation cephalosporin among invasive isolates of *S.pneumoniae* has not been documented yet in Pakistan.[2] Moreover our neighbouring country India has reported just 1.3% penicillin resistance.[7] Therefore there is urgent need to analyse the penicillin and ceftriaxone susceptibility pattern in invasive *S.pneumoniae* isolates so as to guide the physician for empiric use of penicillin, ceftriaxone and vancomycin.

Methods

Study Design
A prospective cross-sectional study was conducted from January 2011 to March 2014 at the Clinical Microbiology Laboratory of Aga Khan University. The Aga Khan Clinical laboratory receives specimens from patients admitted to Aga Khan University Hospital (AKUH), a tertiary care 700 bedded private

setting. In addition, this laboratory receives samples from more than 200 collection points spread all over the country. All invasive strains of *S. pneumoniae* isolated from cerebrospinal and blood were included. Duplicates were also excluded. In case of isolation of *S. pneumonia* from both cerebrospinal fluid (CSF) and blood of same patient, only CSF isolate was included in the study. Patient's demographic data as well as clinical information including drug therapy was gathered via telephonic communication with respective clinicians.

Microbiological Identification and Susceptibility Testing Methods

Identification of *S. pneumoniae* was made using American Society of Microbiology guidelines. Initial suspicion of organism was made on the basis of centrally depressed colony morphology and β-hemolysis, grams staining and catalase test. Final identification was made by conventional tests such as optochin and bile salt susceptibility.[8, 9] To perform susceptibilities, 0.5 McFarland was made than sterile swab was squeezed to remove the excessive fluid, lawn was made on Mueller-Hinton agar with 5% sheep blood (SB-MHA), than E-test strip of penicillin and ceftriaxone was placed over the lawn. Plates were incubated for 24h. After 24h of incubation MICs were measured and interpreted according to breakpoints provided by the Clinical and Laboratory Standards Institute (CLSI) guideline and isolate was categorized as sensitive (S), intermediate (I) or resistant (R).[10] As current CLSI MIC break points for penicillin and ceftriaxone are different for meningeal and non-meningeal isolates. For meningeal strains, penicillin MIC ≤0.06 µg/ml is considered susceptible while MIC of ≥0.12 µg/ml is categorized as resistant. Similarly for meningeal isolates ceftriaxone MIC of ≤0.5 µg/ml, 1µg/ml and ≥2 µg/ml are defined as susceptible, intermediate and resistant.

Data Analysis

Data was coded and entered into SPSS version 21.0 for statistical analysis. Mean and standard deviation of the continuous variables i.e. age, penicillin and ceftriaxone MICs were calculated. Frequency and percentage of the categorical variables i.e. source of specimen, antibiotic susceptibility categories were calculated.

Results

During the study period a total of 146 *S. pneumoniae* were grown from blood and CSF. A total of 46 *S. pneumoniae* were considered meningeal isolates. 42/46 patients belonged to Karachi. Regarding age group of patients, 33% (n=15) belonged to <5 years, 22 (48%) were between 5-65 years of age while 09(19%) were above 65 years of age.

Out of these 46, 27 isolates were from CSF and four of these were from both CSF and blood. From these four cases only CSF isolates were included in the analysis. Nineteen additional isolates yielded from blood cultures were interpreted according to meningeal break-points as clinical history was suggestive of meningitis as shown in table 1.

Table 1: Distribution of *Streptococcus pneumoniae* isolates in clinical specimens.

Meningeal isolates of *Streptococcus pneumoniae* 46 (%)	
Cerebrospinal fluid	19 (41.3)
Cerebrospinal fluid and Blood	04 (8.7)
*Blood	23 (50)

**S. pneumoniae* isolated only from blood but clinical symptoms and signs were suggestive of meningitis

Out of 46 meningeal strains 12(26.1%) isolates were found to be resistant to penicillin while none were resistant to ceftriaxone and vancomycin. Chloramphenicol resistance was found in 6 (14.3%) isolates.

Regarding penicillin and ceftriaxone minimum inhibitory concentration, MIC50 and MIC90 was evaluated as shown in table 2.

Discussion

This study reveals penicillin and ceftriaxone susceptibility of *S.pneumoniae* causing meningitis in Karachi. This data clearly indicates that penicillin resistance is high (26%) in *S.pneumoniae* causing meningitis, however, ceftriaxone resistance was not detected in local isolates. Published data from different countries reports variable prevalence of penicillin and ceftriaxone resistance amongst *S. pneumoniae*. For example study from South Africa reveals penicillin resistance in 33% of their invasive strains[11] while a centre in Baltimore, USA[12] had reported 48.6% penicillin resistant strains. On the other hand neighbouring countries such as India and Bangladesh reported very low prevalence of penicillin non-susceptible isolates in their studies.[13-15] Regarding ceftriaxone non-susceptibility, international data again shows wide variation.[16-18] In Pakistan surveillance data for susceptibility of invasive *S. pneumoniae* isolates is not sufficient. In fact current study comprised of highest number of meningitis causing *S. pneumoniae* isolates that were analysed for susceptibility pattern. A previous community based study evaluated 15 strains of *S. pneumoniae*, from two major cities of Pakistan, Karachi and Hyderabad; the resistance susceptibility of *S. pneumoniae* to penicillin in that study was also 26%, consistent with our findings.[19] In addition we evaluated available antibiogram published by different tertiary care hospitals of Karachi and found very low percentage of penicillin resistance in *S. pneumoniae*.[20]

Current international guidelines recommend empiric vancomycin use along with ceftriaxone for the treatment of pneumococcal meningitis. Out of 46, 12 isolates (26%) revealed resistance to penicillin and justified the need of empiric ceftriaxone in cases of suspected bacterial meningitis. However, as all of the meningitis causing *S. pneumoniae* strains revealed ceftriaxone

Table 2: Penicillin and ceftriaxone susceptibility pattern of *Streptococcus pneumoniae* isolates (n=46) from patients with meningitis

Antibiotic	Susceptibility break point(µg/ml)			No. (%)of isolates(n=46)		
	Susceptible	Intermediate	Resistant	Susceptible	Intermediate	Resistant
Penicillin	≤0.06	--	≥0.12	34(74)	-	12(26)
Ceftriaxone	≤0.5	1	≥2	46(100)	0	0

MIC50 and MIC90 of *Streptococcus pneumoniae* meningeal isolates

Antibiotic	MIC 50	MIC90
Penicillin	0.046	0.125
Ceftriaxone	0.023	0.094

MIC within susceptible range with MIC50 and MIC90 of 0.023, 0.094 µg/ml respectively. Therefore, our finding points out the overuse of empiric vancomycin for *S.pneumoniae* meningitis. Our data also shows that penicillin MIC90 for *S. pneumoniae* isolates was 0.125µg/ml, which further supports sole use of ceftriaxone in our setting. This is based on international treatment guidelines[21] which recommend sole ceftriaxone usage at penicillin MIC of ≤1µg/ml. We suggest that resource limited laboratories; instead of simultaneous performance of penicillin and ceftriaxone MIC, can perform penicillin MIC first and if it is ≤1µg/ml, then do not perform ceftriaxone MIC. In these cases a comment can be released saying that low penicillin MIC indicates susceptibility to ceftriaxone.

This study highlights the importance of use of blood culture for the diagnosis of pneumococcal meningitis. Out of 46 cases, blood culture was found positive in 58.6% cases which indicate that in addition to CSF culture, blood culture should always be requested in suspected cases of *S. pneumoniae* meningitis. In addition, keeping in consideration the difficulty of drawing CSF from very sick patient whom lumber puncture is contraindicated, blood culture can be considered as a safe and high-yield alternative.

In conclusion, penicillin resistance is found in one quarter of *S. pneumoniae* causing meningitis justifying the empiric use of ceftriaxone. However, resistance to ceftriaxone was not seen in this study which suggests that the addition of vancomycin in empiric therapy of pneumococcal meningitis is currently not required in those patients who acquired meningitis locally and have no history of travel. Continuous monitoring of antimicrobial susceptibilities is required by clinical laboratories to find out any emerging ceftriaxone resistance.

Conflict of Interests

None to declare

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ORIGINAL ARTICLE

Yellow Babies - An Index of Suspicion for Urinary Tract Infection

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Abstract

Background
Neonatal jaundice has long been recognized as a commonest problem in neonate. Most of the time, it is benign. In young infant's bacterial urinary tract infection (UTI) can lead to serious outcome. Clinical presentation of UTI in neonates has variable spectrum ranging from asymptomatic to severe sepsis. We performed this study to determine the incidence of neonatal jaundice among sick neonates who developed UTI in a tertiary care center.

Material & Method
We performed this cross-sectional study at neonatal intensive care unit and neonatal high density unit (HDU) of pediatric medicine of Liaquat National Hospital and Medical College over a period of one year (January 2013 - December 2013). The total sample size of our study was 173 neonates. Incidences of UTI (on the basis of urine direct report and culture) as well as most common organisms leading to UTI were recorded.

Results
Of the173 patients enrolled in the study, 82 (47%) were male and 91(52%) were female. A total of 77 out of 173 (45%) jaundiced neonates were diagnosed to have pyuria (based on positive urine direct report), of those 30 (39%) patients had a positive urine culture. The most common bacterial pathogen isolated was *E.coli* (43%), followed by *Klebsiella* (27%), *Acinetobacter* (13%), *Proteus* (10%) *and Staphylococcus aureus* (7%) respectively. Moreover, only 25 (14%) neonates had predominantly direct hyperbilirubinemia while the rest had indirect hyperbilirubinemia (86%).

Conclusions
UTI may present with jaundice in neonates, thereforeany neonate who presents with pathological jaundice should be investigated for urinary tract infections.

Key Words
Neonates, Urinary Tract Infection, Jaundice.

Introduction
Neonatal jaundice in most cases recognized as a benign problem for long time.[1] It is considered as common cause of newborn admission in neonatal units incidence is 39.7/1000 live birth.[2] During the first week of life 80% preterm and 60% term develop visible jaundice.[3, 4]

In young infant urinary tract infection (UTI) is a serious bacterial infection. Its incidence varies from 0.1 – 1% in neonates and from 5% and 11% among febrile infants.[5] In neonates, clinical presentation of UTI is extremely variable ranging from asymptomatic to severe sepsis.[6] The first sign of UTI in asymptomatic infants may be jaundice before other signs and symptoms become obvious. Jaundice was found in 6.8% neonates with urinary tract infection.[7]

Accumulation of unconjugated, nonpolar, lipid-soluble bilirubin pigment in the skin lead to yellow color of jaundice.[8] The antioxidant nature of bilirubin is very protective and it is said to protect the antioxidant deficient baby from oxygen toxicity in the early neonatal life.[9]

Some cases of otherwise benign physiological jaundice may have other diseases in combination. Variousresearches has been done on the association of idiopathic hyperbilirubinemia and bacterial infections, such as urinary tract infection (UTI). Relation of coincidental UTI and hyperbilirubinemia is still ambiguous. However, some theory proposed the idea of that gram negative bacillus especially *E.coli* produces hepatotoxins which leads to hemolysis of RBCs causing indirect hyperbillirubinemia. Though direct hyperbillirubenemia with UTI may be due to cholestasis, for which exact etiology is still unknown.[7]

There is currently no recommendation for UTI evaluation among neonates with neonatal hyperbilirubinemia. Urinalysis and a urinary culture are only recommended under some certain conditions, such as infants who have an elevated conjugated bilirubin, and infant readmitted for phototherapy or exchange transfusion.[8] However, the coincidental occurrence of UTI with indirect hyperbilirubinemia in our clinical practice supported by some studies prompted us to undergo this research so as to emphasize upon routine investigation of icteric babies for UTI.

Material & Methods
This was a cross-sectional study conducted in the NICU and HDU of Department of Pediatric Medicine of Liaquat National

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Hospital and Medical College over a period of one year, from January 2013 till December 2013.The inclusion criteria included all the neonates (age = 28 days) admitted in NICU with complain of neonatal jaundice irrespective of gestational age and weight.

The exclusion criteria included all the babies (age > 28 days) and all those who are admitted with complain other than jaundice. A questionnaire was made which was kept in Neonatal Intensive Care Unit (NICU) and HDU.It was filled by the duty doctor and was then checked by the researcher. Demographic and historic factors such as gender, gestational age, body weight, mode of feeding, circumcision status and onset of jaundice were recorded. Blood samples were taken from all the cases and their complete blood count with reticulocyte count ,serum bilirubin level (total and direct), coomb's test, G6PD level, maternal and neonatal blood group were evaluated.

In addition, urine analysis and urine cultures were performed. Furthermore, the enrolled patients were divided intogroups, with and without pyuria according to the results of urinalysis reports.

Definite pyuria was defined as leukocyte count of 20/HPF. Cases with UTI were defined as the presence of any number of colony forming units (CFU)/mL of pathogens obtained by supra-pubic puncture collection, more than 10,000 CFU/mL obtained by bladder catheterization, or more than 100,000 CFU/mL of pathogens obtained by urinary bag collection. Otherwise, the patients would be grouped into the no UTI group.

After initial evaluation and treatment of the infants ultrasound were requested to all cases with UTI. The data was analyzed using SPSS software.

Significant Values:-Descriptive data were reported as mean ± SD. Statistical significance was defined as P value < 0.05.

Results

Of the173 patients enrolled in the study, 82 (47.4%) were male and 91(52.6%) were female. All males were uncircumcised. The mean gestational age of all neonates was 38.6 weeks range (37-41weeks) and mean body weight was 3.32 kg range (2.61-4.25kg).A total of 77 out of 173 (44.5%) jaundiced neonates were diagnosed to have pyuria (based on positive urinalysis).

Table 1 shows that 77 babies had pyuria out of which 30 patients had a positive urine culture. More females (61%) than males (38.9%) had pyuria and positive urine culture (M=43.3%, F=56.6%).

The most common bacterial pathogen isolated was *E.coli* (43.3%), followed by *Klebsiella* (26.6%), *Acinetobacter* (13.3%), *Proteus* (10%) and *S.aureus* (6.6%) as shown in Fig.1.

Work up revealed physiological jaundice to be the commonest cause of hyperbilirubinemia. UTIs were observed more commonly in bottle-fed infants and infants with mixed feeds than breast-fed infants as shown in Fig. 2. There was no significant difference between neonates with UTI and without UTI in respect to gestational age, body weight and total serum bilirubin (TSB) at the time of admission.

Table 1: Distribution of neonates according to urinalysis & urine culture results.

	Pyuria	No pyuria
Male	30	52
Female	47	44
Total	77	96
Pyuria present	**Positive urine culture**	**Negative urine culture**
Male	13	17
Female	17	30
Total	30	47

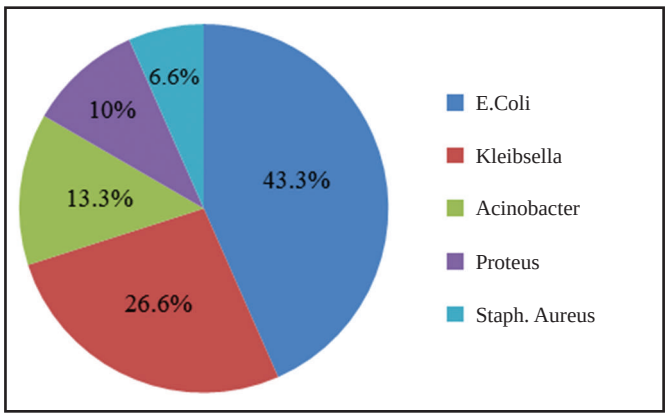

Fig 1. Frequency of isolated bacterial pathogen in urine culture.

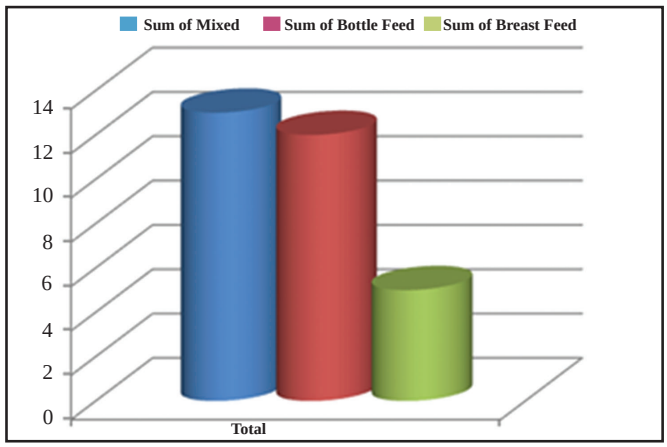

Fig 2. Number of neonates with UTI and their mode of feeding.

Table 2 shows 143 (82.6%) of neonates had jaundice reported before or at 08 days of age and 30(17%) after 08 days. Moreover, of 173 jaundiced babies, only 25 babies had predominantly direct hyperbilirubinemia (14.4%) while the rest had indirect hyperbilirubinemia (85.5%).

The renal ultrasound revealed urinary tract abnormalities in only 2(1.15%) patients which was hydronephrosis, while grade II parenchymal changes were shown in 13(7.5%) patients.

Table 2: Distribution of neonates according to onset and type of jaundice.

	Age less than 8 days of life	Age between 8-14 days of life
Onset of jaundice	143	30
Type of hyperbillirubenemia	**Direct** 25	**Indirect** 148

Discussion

The results of our study revealed that 44.5% of the jaundiced babies had pyuria, of which 39 % were culture positive and the most common organism isolated was *E. coli*. Presence of UTI in this significant number of jaundiced patients is an important issue and needs to be emphasized so that proper diagnosis could be established and appropriate management be instituted.

Association of UTI with hyperbilirubinemia is not a new topic for the researchers. It was mentioned in studies about eight decades back and again for the last four decades it has been re-emphasized. It has been known for long that patients with UTI usually exhibit conjugated hyperbilirubinemia[9, 10] but our study shows contrary to this fact, almost all the babies presented with unconjugated hyperbilirubinemia. This could probably be due to the fact that higher levels of bacteremia could produce a higher rate of hemolysis and, consequently increased unconjugated bilirubin levels.

The incidence of UTI was seen to be higher in our study as compared to the studies by Garcia & Nagar, Bilgen, Chavalitdhamrong, Hung-Ta Chen *et al*, and Tariq Khudair and Hussain (E.coli7.5%, *Kleibsella*8%,*Acinobacter* 2.9%, *Proteus*14%, and*S. aureus* 6.5% respectively).[3,9,10,11]

Similar to other studies body weight and gestational age were not associated with UTI. In contrast to the results of other studies,[12] urinary tract abnormalities were seen in only 1.15% of neonates with UTI, this difference may be due to the smaller number of patients with UTI.

In most of the studies, conjugated hyperbilirubinemia predominated in babies with UTI and this was quite justified by various mechanisms like "microcirculatory changes in the liver, direct effect of bacterial products and or endotoxin induced mediators".[13] However in our study majority of the babies with UTI had indirect hyperbilirubinemia. This could be well explained by the fact that the bacterial burden and toxins can cause higher rate of hemolysis and hence can lead to unconjugated bilirubin levels[14]. Moreover, cholestasis can also occur due to infection and possible low activity of the enzyme glucuronictransferase may contribute to the high levels of indirect bilirubin in these neonates.[15,16]

Routine screening for UTI in asymptomatic jaundiced babies is still controversial. According to American Academy of Pediatrics' guidelines; for all newborn infants 35 or more weeks of gestation, it is recommended that the clinicians should perform urinalysis in the presence of increased direct bilirubin levels or readmission for phototherapy or exchange transfusion.[8] Additional laboratory evaluation for sepsis should be performed if indicated by history and physical examination. However, in our study, UTI in babies presented with indirect hyperbilirubinemia. Similar findings have been seen in the study by Lee *et al*.[17] Therefore, we strongly suggest that urinary tests for UTI should not only be confined to babies with conjugated hyperbilirubinemia. People have argued against the routine testing as more than two thirds of normal infants especially in Asians present with jaundice during first weeks of age and the reported incidence of UTI in jaundiced infants varied from 1% to 11% in different countries.[3, 18, 19.]

Irrespective of the mechanism and type of hyperbilirubinemia, it seems justifiable to investigate all asymptomatic babies with jaundice for UTI. Urine analysis seems cost effective even in resource poor settings like ours when there's a benefit of treating UTI versus risk of missing it.

Conclusion

Jaundice is a common presenting symptom in neonates; its association with UTI has not been established and amplified globally. In our study we have found that UTI may present with pathological jaundice in neonates, so we conclude that all neonates presenting with jaundice should be investigated for urinary tract infections.

Conflict of Interest

The authors whose names are listed above certify that they have no affiliations with or involvement in any organization or entity with any financial interest (such as honoraria; educational grants; participation in speakers' bureaus; membership, employment, consultancies, stock ownership, or other equity interest; and expert testimony or patent-licensing arrangements), or non-financial interest (such as personal or professional relationships, affiliations, knowledge or beliefs) in the subject matter or materials discussed in this manuscript.

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ORIGINAL ARTICLE

Frequency of Extended Spectrum Beta Lactamases Producing Gram Negative Bacilli among Clinical Isolates in Tertiary Care Hospital at Wah

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Abstract

Background
This study was carried out to determine frequency of extended spectrum beta lactamases producing Gram negative bacilli among clinical isolates as a result of culture of all specimens received in POF Hospital laboratory at Wah.

Material and Methods
Three hundred and eighty five clinical isolates of Gram negative rods from various clinical specimens in six months period from September 2010 to Feb 2011 were included in the study. All the isolates were processed by standard microbiological methods. The detection of extended spectrum beta lactamase (ESBL) production in these isolates was carried out by double disc synergy method.

Results
Out of three hundred and eighty five isolates, ninety three (24.2%) were ESBL producers and two hundred and ninety two (75.8%) were non ESBL producers. *Escherichia coli* was the most commonly isolated ESBL producer followed by *Klebsiella pneumoniae.*

Conclusion
ESBL producing isolates are prevalent in our setting. Infection control measures and judicious use of extended spectrum antibiotics are required to combat this grave situation.

Keywords
Extended spectrum beta lactamases, Gram negative bacilli, Extended spectrum antibiotics.

Background
The world of infectious disease has become very complicated due to antimicrobial drug resistance which is an inevitable consequence of injudicious use of antimicrobial therapy.[1] Extended spectrum beta lactam antibiotics such as third generation cephalosporins form the major component of the empirical antibacterial armamentarium in the most clinical setups. In the past it was believed that cephalosporins were relatively immune to attack by β-lactamases.[2] The persistent exposure of bacterial strains to a multitude of β-lactamas has induced a dynamic and continuous production and mutation of β-lactamases in these bacteria, expanding their activity even against the third generation cephalosporins. Thus these enzymes are called extended spectrum β-lactamases.

However, there is no consensus on the precise definition of ESBL. A commonly used working definition is that extended-spectrum β-lactamases are a rapidly evolving group of β-lactamases which share the ability to hydrolyze third-generation cephalosporins and aztreonam but are inhibited by clavulanic acid.[3]

Important ESBL producing Gram negative bacilli are *Escherichia coli, Klebsiella pneumonia, Proteus mirabilis, Enterobacter* spp., *Citrobacter* spp., *Pseudomonas aeruginosa and Acinetobacter baumannii.*[4] The frequency and susceptibility pattern of ESBL producing organisms differ significantly in accordance with geographical locations.[5] There exists marked hospital to hospital variability even within a country.[6] The variable pattern of ESBL producing isolates parallels the misuse or overuse of beta lactam drugs.[7] Frequency of ESBL producing Gram negative bacilli is 28% in Bulgaria, 16% in Cyprus and Romania, 12% in Portugal.[8] Studies in India showed a frequency of 20%[9] while Ali *et al*[4] and Jabeen et al[10] from Pakistan have reported a frequency of 43% and 50% respectively from Rawalpindi and Karachi. The available therapeutic options for the treatment of ESBL producers associated infections are limited by drug resistance conferred by ESBL producing organisms, along with frequently observed cross resistance to other antibiotics as well, like aminoglycosides, quinolones and cotrimoxazole.[10]

Detection of ESBL production is of utmost importance both in hospital and community isolates. The prevalence of these enzymes is increasing worldwide with rates varying greatly even among hospitals in a given region.[11] The ESBL producers constitute a serious threat to currently available antibiotics, as they leave behind limited therapeutic choices. The institutional outbreaks are increasing because of selective pressure due to excessive use of extended spectrum cephalosporins and lapses in effective control measures. Extreme vigilance and timely recognition of infections with ESBL producers and appropriate

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antibiotic therapy is the only answer to the current grave situation.

Frequency and susceptibility pattern varies from region to region and time to time, so local pattern of susceptibility or institutional antibiograms should be used to determine the choice of drug.[12] Indiscriminate use of antibiotics lead to emergence of multidrug resistant ESBL producing Gram negative pathogens and nosocomial outbreaks that could bring about prolonged stay, increased mortality, expensive control efforts and therapeutic failures.

The objective of this study is to document the frequency of ESBL producing Gram negative bacilli in tertiary care hospital at Wah bearing in mind that no such study had been carried out before. This effort will limit inappropriate antimicrobial usage and rationalize empirical therapy to avoid antibiotic selective pressure along with improvement of infection control measures focusing on reducing patient to patient transmission via the inanimate environment, hospital personnel and medical equipments, hence decrease emergence and dissemination of antimicrobial resistance in our setup.

Materials and Methods

This descriptive study was carried out in Microbiology section of Pakistan Ordnance Factories Hospital laboratory from September, 2010 to February, 2011 on clinical samples received from admitted patients as well as patients from outdoor departments. The specimens were inoculated on appropriate culture medium like blood agar, MacConkey agar, chocolate agar (sputum) and cysteine lactose electrolyte deficient agar (urine). These were incubated at 35-37°C under aerobic conditions for 24 hours. After overnight incubation, the agar plates were examined for growth of bacteria and their colonial morphology. The Gram negative rods were identified based on Gram staining and biochemical tests.[13] Antimicrobial susceptibility tests were performed on the Muller–Hinton agar plates with disk diffusion method as recommended by clinical laboratory standards institute.[14]

Double disk synergy method was used to screen ESBL producing isolates. After subculture of suspensions of the isolates on Mueller-Hinton agar , an antibiotic disk containing amoxicillin-clavulanate (20/10 µg) was placed in the centre of the plate. Disks of 30 µg aztreonam, ceftazidime, ceftriaxone and cefotaxime were placed at a distance of 30 mm (edge to edge) from the central disk. Zones of inhibition around the third generation cephalosporin disks and aztreonam were observed after overnight incubation at 37°C. The organism reflecting inhibition zone around one or more cephalosporins disks and aztreonam extended on the side nearest to amoxicillin-clavulanate was considered to show the synergism and it was identified as an ESBL producer.[15]

E. coli ATCC 25922 and *K. pneumoniae* ATCC 700603 were used for quality control of the test procedure.[14]

The data was entered and analyzed using SPSS version 10. For qualitative variables (ESBL producers among Gram negative bacilli, gender, type of samples and organisms isolated) frequencies and percentages were calculated. Mean ± SD was presented for age.

Results

A total of three hundred and eighty five (385) isolates of Gram negative rods were studied. Majority of isolates (*n*=163, 42.3%) were isolated from urine. The other isolates were from blood (17.7%), high vaginal swabs (11.4%), pus (10.4%), catheter tips (6%), ear swabs (5.5%), body fluids (1.8%), endotracheal tubes (1.6%), sputum (1.6%), tracheostomy discharge (1.3%) and throat swabs (0.5%). The distribution of specimens is presented in table 1.

Out of three hundred and eighty five (385) isolates, ninety three (24.2%) were ESBL producers and two hundred and ninety two (75.8%) were non ESBL producers. The commonest ESBL producing organism isolated was *Escherichia coli* (47.3%), followed by *Klebsiella pneumoniae* (24.7%), *Pseudomonas aeruginosa* (8,6%) and *Enterobacter aerogenes* (7.5%). (Table-2)

Out of ninety three ESBL producer isolates, 54.8% were recovered from female patients and 45.2% from male patients.

Mean age of the patients was 47.38 + 24.22 years (mean + SD). Age distribution of different age groups which yielded ESBL isolates is shown in Figure 1.

Thirty seven isolates (39.8%) were isolated from outdoor patients, while the remaining (*n*=56, 60.2%) were from patients admitted in different wards including General Medicine (29%), Medical intensive care unit (9.7%), General surgery (8.6%), Neonatal intensive care (5.4%), Paediatrics Medicine (4.3%), Gynecology/obstetrics (2.2%) and ENT ward (1.1%). The distribution is presented in Figure 2.

Discussion

Antibiotic resistance surveillance has a key role among all strategies to manage antibiotic resistance as an inevitable consequence of indiscriminate and injudicious utilization of antimicrobial therapy. There is tremendous variability of antimicrobial resistance in different geographic regions. This phenomenon makes continuous surveillance of the extent and trends of antimicrobial resistance essential for guiding effective empiric therapy in every continent, country, city, hospital or even health care unit. [16] This study had provided an overview of the current situation regarding ESBL producing Gram negative bacilli in POF hospital with a focus on their frequency. In our study, the frequency of ESBL producer isolated among Gram negative bacilli was 24.2%. *Escherichia coli* (47.3%) was the most commonly isolated pathogen followed by *Klebsiella pneumoniae* (24.7%) and *Pseudomonas aeruginosa* (8.6%).

Table 1: Frequency of different types of clinical specimens (n=385)

Specimens	Frequency	Percent	Valid Percent	Cumulative Percent
Blood	68	17.7	17.7	17.7
Tracheostomy discharge	5	1.3	1.3	19.0
Endotracheal tubes	6	1.6	1.6	20.5
Urine	163	42.3	42.3	62.9
High viganal swabs	44	11.4	11.4	74.3
Sputum	6	1.6	1.6	75.8
Catheter tips	23	6.0	6.0	81.8
Biological fluids	7	1.8	1.8	83.6
Pus	40	10.4	10.4	94.0
Throat swabs	2	0.5	0.5	94.5
Ear swabs	21	5.5	5.5	100.0
Total	**385**	**100.0**	**100.0**	

Table 2: Frequency of ESBL producers among Gram negative isolates (n=93)

	Frequency	Percent	Valid Percent	Cumulative Percent
Escherichia coli	44	47.3	47.3	47.3
Serratia marcescens	2	2.2	2.2	49.5
K.pneumoniae	23	24.7	24.7	74.2
Enterobactor aerogenes	7	7.5	7.5	81.7
Acinetobacter baumannii	2	2.2	2.2	83.9
Pseudomonas aeruginosa	8	8.6	8.6	92.5
Proteus mirabilis	2	2.2	2.2	94.6
Proteus vulgaris	3	3.2	3.2	97.8
Citrobacter ferundii	2	2.2	2.2	100.0
Total	**93**	**100.0**	**100.0**	

Analysis of studies over the last decade had revealed frequencies of 40-50% of ESBL producer in different parts of Pakistan. Shah et al had reported a frequency of 48% of ESBL producer with *E. coli* as the most prevalent organism among the patients of age group ranging from 50-60 years. ESBL producing isolates were mostly reported in males (65.3%) compared to females (34.7%).[17] These findings were in contrast to our results which showed increased frequency of ESBL producing isolates among female patients as compared to males. Whereas the patients of age group ranging from 61 to 70 years have yielded maximum ESBL producing isolates.

In a study carried out in Karachi, 40% of Gram negative isolates were found to be ESBL producers. ESBL positivity was detected in 50% *Enterobacter* spp., 42% *E. coli* and 36% *K. pneumoniae*. ESBLs producing Gram negative bacilli were more frequent in nosocomial isolates and in patients at extremes of ages (under 5 years and more than 60 years).[10] The later findings were similar to our study in which majority of ESBL producers were of hospital origin and in patients at extreme of age.

Ali *et al* had reported a frequency of 45% of ESBL producing Gram negative isolates from Rawalpindi with *Enterobacter cloacae* (79%) as the most prevalent organism followed by *Acinetobacter baumannii* (72%) and *Klebsiella oxytoca* (66.7%).[18] These bacilli were different from those which were yielded in our setup, thus reflecting variable pattern of ESBL producing isolates.

There are studies which show changing trends in frequency of ESBL producing Gram negative bacilli. Sattar et al reported decreased frequency of Gram negative bacilli which were ESBL producers during four years (2003-2007).[19] It was 66% during December 2003- November 2004, 54% during December

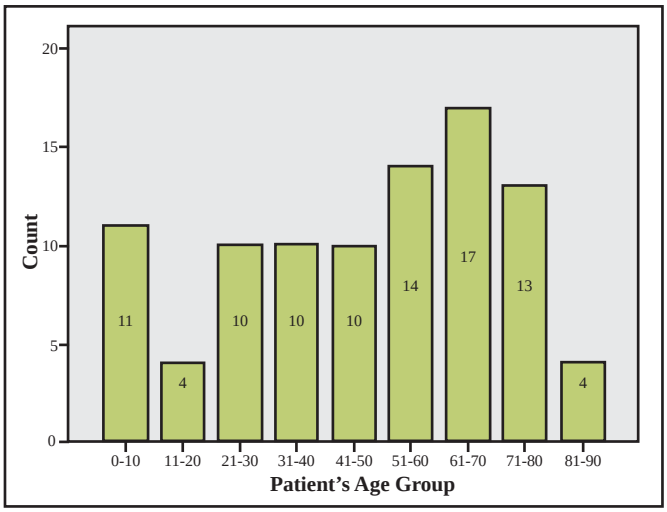

Fig 1. Age Distribution of patients infected with ESBL producing isolates (n=93)

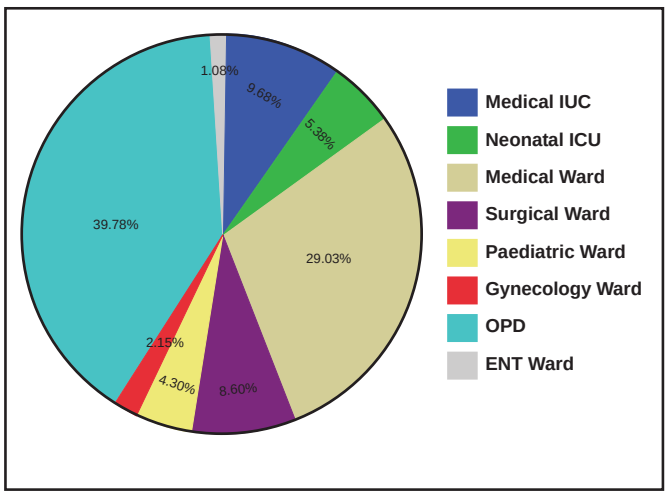

Fig 2. Ward / OPD distribution of ESBL producing isolates *(n=93)*

2004- November 2005), 57% during December 2005- November 2006 and 47% during December 2006- November 2007. The study concluded a decrease trend in the frequency of ESBL producers but the organisms showing same resistance pattern as ESBLs producing isolates were on rise.

The results of our study were closer to study carried out in Lahore which revealed 35.5% of bacterial strains to be ESBL producers. The commonest ESBL producing organisms isolated were *E. coli* (44.8%), followed by *K. pneumoniae* (38.6%), *P. mirabilis* (31.6%) and *A. baumanni* (7.1%).[1] Similarly, study conducted by Ullah et al to assess ESBL prevalence in *Pseudomonas aeruginosa* isolated from burn patients in the North West of Pakistan revealed 35.85% isolates to be ESBL producer.[20] The high percentage of ESBL producers reflected the extensive and indiscriminate use of antimicrobials in our setup.

Comparison with studies which were conducted in other parts of the world revealed that the highest isolation rate of ESBLs producing *K. pneumoniae* has been reported from the Latin America (54.4%), the Western Pacific (24.6%) and Europe (22.6%). The frequency of ESBL producing *E. coli* in this area was reported to be 8.5% , 7.9 % and 5.3% respectively. [21] Studies from India also revealed variable incidence of ESBL producers. Roopa et al conducted study on the most prevalent uropathogens which revealed 87.5% of E.coli and 66.7% of *K.pneumoniae* to be ESBL producers.[22] Earlier, Taneja et al reported that ESBL producers could explain only 36.5% of multi drug resistant uropathogens in their study.[23] Similarly Aladag et al conducted a study on *K.pneumoniae* isolated from urinary tract infection. Among 125 isolates of *K.pneumoniae*, 45 strains (36%) produced ESBL.[24] In our study frequencies of 47.3% and 24.7% were found for *E. coli* and *K. pneumoniae* respectively which were closer to the results seen in other studies.[23,24]

A study in Iran reported 21% of isolates of *E.coli* and 12% of *K.pneumoniae* to be ESBL producers. Of 150 patients who had positive ESBLs isolates, 104 (69.3%) were outpatients and 46 (30.6%) were hospitalized.[25] This finding was in contrast to our study in which most of ESBL producing organisms were hospital acquired.

The frequency of 24.2% ESBL producing isolates in present study showed slight decreased trend as compared to other studies.

We have used the commonly employed double disk diffusion method for detection of ESBL. Evaluation of the double disk synergy method against strains which have been genotypically confirmed to be ESBL producers or non producers have revealed sensitivities of the method ranging from 79% to 97% and specificities ranging from 94% to 100%.[26, 27]

Conclusions

Our study revealed *E.coli* followed by *K.pneumoniae* as the most frequent ESBL producing Gram negative bacilli. These isolates were found predominantly in hospital settings, in extremes of age and in females. To conclude, ESBL producing isolates are prevalent in our setting.

Recommendations

Considering the grave scenario of antibiotic resistance in our country, it is high time that clinical laboratories should detect the ESBLs routinely and accurately and the clinician should reformulate the antibiotic policies, so that rationalized therapy can be instituted to avoid injudicious use of antibiotics.

The methods for detection of ESBL should be improved to assure the possibility of interventions in time.

Significance of national public health efforts should be high

lightened to implement surveillance, epidemiologic, environmental health and policy making components.

Infection control measures are recommended, so that appropriate management can be instituted and spread of these organisms curtailed.

The use of extended spectrum cephalosporins should be limited to cases in which other therapeutic alternatives according to evidence-based guidelines are not possible.

Competing Interests

The authors declare that we have no conflicting interest.

Authorship Declaration

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.

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ORIGINAL ARTICLE

Neonatal Sepsis: An Evaluation of Bacteriological Spectrum and Antibiotic Susceptibilities in NICU of Children Hospital Multan

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Abstract

Objective
Neonatal sepsis is a clinical syndrome of both infection and systemic inflammatory response in the first month of life. The microbiological spectrum and their antibacterial sensitivities vary across different communities. We performed this study to determine the causative pathogen and antibiotic susceptibility of neonatal sepsis in our neonatal unit to design local neonatal sepsis management guidelines.

Method
This descriptive cross-sectional study was undertaken at neonatal intensive care unit of Children Hospital and Institute of Child Health, Multan using nonprobability convenience sampling technique from Jan-Dec 2014.All neonates (from birth till 28 days) admitted with clinical signs and symptoms suggestive of sepsis were included in the study, excluding neonates with congenital anomalies and history of previous antibiotics administration. Blood cultures were drawn and processed using standard protocols. We calculate early onset and late onset neonatal sepsis incidence; common pathogens and their antibacterial sensitivities associated with these neonatal sepsis.

Results
Of 161 total patients, 107 (66%) were male and 54(44%) were female. Early onset sepsis (EOS) was present in 98 (61%) cases and late onset sepsis (LOS) in 63 (39%).Gram negative isolates were 65(61%) and Gram positive were 42(39%).Among Gram negative organisms, *Pseudomonas aeruginosa* was most frequent,(n=22, 34%) and in Gram positive isolates, *Staphylococcus epidermidis* was the most frequent, (n=18, 43%).The Gram positive isolates showed 100 % sensitivity to linezolid and 100% resistance to ampicillin and Gram negative showed 85% sensitivity topipercilliin/tazobactum and 100% resistance to Cefotaxime.

Conclusion
Gram negative microorganisms were more common than gram positives. Isolated bacteria showed high resistance to commonly used antibiotics.

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Key Words
Neonatal Sepsis, Earlyonsetsepsis, lateonsetSepsis, Antibiotic susceptibility.

Background
Neonatal sepsis is a clinical syndrome of both infection and systemic inflammatory response syndrome in the first month of life.[1]If present before 3 days of life, it is early onset sepsis(EOS) and after 3 days to 28 days of life is late onset sepsis(LOS).[2] Of the estimated 130 million annual births worldwide, 4 million die in the first 28 days of life, and nearly half of them on the first day.[3] World Health Organization estimates that 35% of 4 million neonatal deaths (around one and a half million) annually are due to infection.[4] Millennium Development Goal-4 aimed to reduce under-5 mortality (U5M) close to 30 child deaths per 1000 live births by 2015[5] but lacked behind and could achieve only 48 deaths per 1000 live births largely through interventions to lower mortality after the first month of life and neonatal survival was not addressed properly that's why neonatal mortality rate (NMR) is still 44% of the total child mortality.[6]

Every Newborn Action Plan(ENAP) now aiming to reduce NMR below 10 per 1000 live births till 2035[6] and Pakistan is one of its partners as it is amongst the top 8 countries with highest neonatal mortality (55/1000 live births).[7] Neonatal sepsis is the third most important cause of neonatal mortality after prematurity and birth asphyxia.[8] Incidence of neonatal sepsis varies from 1 to 5 cases per 1000 live births in developed countries, but gets higher in developing countries which varies from 49 to 170 per 1000.[8]

Pathogens causing neonatal infection or sepsis differ between countries; institutions within the same country and even within the city and so are the sensitivity pattern to commonly used antibiotics due to local protocols.[9] There is lack of local published literature on common EOS and LOS pathogens and their susceptibility pattern. We designed this descriptive cross sectional study to determine common causative bacterial pathogen in neonatal sepsis along with their susceptibility pattern to antimicrobial drugs in neonates admitted to neonatal intensive care unit (NICU) of children hospital complex Multan, Punjab, Pakistan.

Material and Methods

This descriptive cross-sectional study was conducted at neonatal intensive care unit (NICU) of Children Hospital and Institute of Child Health Multan from 1st January 2014 to 31st December 2014. Our NICU is 20 bedded including 8 incubators and 8 ventilators. Annual neonatal admissions through outpatient and emergency departments are 6000 and 646 shifted to NICU where annual deaths are 281. First line antibiotics are Ceftazidime and Amikacin.

A total of 256 neonates of either gender (from birth till 28 days) admitted with clinical signs and symptoms suggestive of sepsis i.e fever, grunting, cyanosis, respiratory distress, reluctance to feed, vomiting, lethargy and weak cry, were included in the study after informed consent from their parents. We performed non probability convenience sampling. Blood culture was sent in these cases. A single specimen of 2 to 3 ml of blood was collected from peripheral vein keeping all aseptic measures. Neonates who received antibiotics prior to admission or had chromosomal or congenital anomalies were excluded from the study. Neonates presenting before 3 days of life were labeled as early onset sepsis and after 3 days to 28 days of life were as late onset sepsis.[2]

161 neonates of either gender were studied after excluding 95 due to either congenital anomalies or prior antibiotic intake in this illness. Detailed history and complete physical examination was carried out on each patient. Blood samples were inoculated on standardized media and incubated at 37ºC for 3 days. Sub-cultures were then made on blood agar, chocolate agar and McConkey agar. The colonies isolated, were identified by their colonial morphology, gram's stain and conventional biochemical and serological tests. Antibiotic susceptibility pattern of the isolates was studied by using conventional disc diffusion technique. Antibiotics relating to culture sensitivity were categorized and analyzed according to their mode of action into groups like Penicillin, Aminoglycosides, Cephalosporins, Fluoroquinolones, Vancomycin, Carbapenems and Linezolid. Other investigations including relevant hematological, biochemical and radiological were also performed. Data was entered, analyzed by SPSS version 19. Descriptive statistics were used to describe the frequency and percentages of different microorganisms in culture positive patients.

Result

Of 161 total patients, 107 (66%) were male and 54(44%) were female. EOS was present in 98 (61%) cases whereas LOS was found in 63(39%) cases. No growth found in 54(33.5%), 42(26%) were Gram positive and 65(40%) were Gram negative.(table1).

Frequent organisms found were *Pseudomonas aeruginosa, 22* (13.8%), *Staphylococcus epidermidis*, 18(11.4%), and *Escherichia coli (E coli)*, 15(9.4%). *Pseudomonas aeruginosa* was most frequent Gram negative organism 22 (33.8%), (table 2), and *Staphylococcus epidermidis* was the most frequent gram positive 18 (42.8%). (table 3).

Table 1: Bacterial isolates responsible for neonatal sepsis in NICU of Children Hospital, Multan

Bacterial Isolates	Frequency(n)	Percentage (%)
No growth	54	34
Gram Negative	65	40
Gram Positive	42	26
TOTAL	161	100

Gram positive isolates had a high resistance (100%) to Ampicillin and Cefotaxime except *Staphylococcus Epidermidis* and *Staphylococcus aureus* which showed 85% to Ampicillinand Cefotaxime respectively. The resistance of Gram negative microorganism to Amikacin was also found very high ranging from 50% by *Salmonella* to 100% by *Proteus* and *Klebsiella*.

In Gram negative isolates *enterobacter, Pseudomonas, klebsiella* and *Proteus* were 100% sensitive to Piperacillin/Tazobactam while *Escherichia coli* and *Salmonella* showed 50 % sensitivity. *Salmonella, Klebsiella* and *Proteus* were 100% sensitive to Cefoperazone/Salbactem while *Escherichiacoli, Pseudomonas* and *Enterobacter* showed 90%, 65% and 30% respectively. (table 4).

Table 2: Gram negative isolates responsible for neonatal sepsis in NICU of Children Hospital, Multan

Gram negative Isolates	Frequency(n)	Percentage (%)
Pseudomonas aeuriginosa	22	34
Escherichia coli	15	23
Enterobacter	8	12
klebsiella	7	11
Salmonella	7	11
Proteus	6	9
Total	**65**	**100**

Table 3: Gram positive isolates responsible for neonatal sepsis

Gram negative Isolates	Frequency(n)	Percentage (%)
Staph epidermidis	18	42
Staph aureus	12	29
Staph saprophyticus	12	29
Total	**42**	**100**

All the isolated Gram positive bacteria were 100 % sensitive to linezolid. *Staphylococcus aureus* and *Staphylococcus epidermidis* developed 75% and 69% resistance to *Imepenem* respectively, while the sensitivity of *Staphylococcuss aprophyticus* is still 100 % to *Imepenem* and 50 % to *Meropenem*. *Staphylococcuss aprophyticus* also showed 100% sensitivity to Piperacillin Tazobactum while *Staphylococcus aureus* and *Staphylococcus epidermidis* showed 50% sensitivity. (table 5)

Discussion

Estimated 4 million neonatal deaths occur worldwide and developing countries are responsible for 99% of these.[5] Worldwide, infections (sepsis, pneumonia, diarrhea and tetanus) are third most important cause of mortality preceded by prematurity and birth asphyxia.[6] NMR (neonatal mortality rate) in Pakistan is 55/1000 live births and infections are 2nd most common cause after prematurity.[10]

Studies state that male neonates are more susceptible to neonatal sepsis compared to females due to reasons not completely understood. Our study also depicted that male are more prone to sepsis than female gender with a ratio of 1.9:1. This finding is consistent with certain studies where male to female ratio was around 2:1.[8, 9, 11, 12]

Although blood culture is gold standard for diagnosis of sepsis but it has limited sensitivity and specificity and yields positive result in only 10-60% of cases.[13] Our study had culture positivity rate of 66.5%, which is around top of this range and if we compare to others in Pakistan i.e. 62.8% by Rahman *et al*,[14] 32% by Ahmad *et al*[15] and17.1% by Goheer *et al*.[16] Late onset sepsis (LOS) is more common as compare to early onset sepsis (EOS) in developed countries[17, 18] which is opposite in developing countries where EOS is more than LOS and we observed similar results in our study where EOS is 61% while LOS is 39% and Sheikh *et al*, Ahmad *et al* and Waseem *et al* reported coinciding results.[10, 15, 19]

Table 4: Sensitivity of Gram negative microorganisms to commonly used antibiotics

Antibiotics	Sensitivity					
	Enterobacter	*Escherichia. Coli aeuriginosa*	*Pseudomonas*	*Klebsiella*	*Proteus*	*Salmonella*
Ampicillin	-	0	-	-	0	-
Amikacin	35	20	33	0	0	50
Ceftazidime	0	0	0	0	0	0
Pipercillin/ Tazobactum	100	40	100	100	100	50
Cefoperazone/Salbactem	70	10	35	100	100	100
Imepenem	70	60	41	100	100	50
Meropenem	50	10	35	100	50	100
Ciprofloxacin	65	10	17	50	50	50

Table 5: Sensitivity of Gram positive microorganisms to commonly used antibiotics

Antibiotics	Sensitivity of Gram positive microorganisms to commonly used antibiotics		
	Sensitivity of Staphylococcus aureus %	*Sensitivity of Staphylococcus epidermidis* %	*Sensitivity of Staphylococcus saprophyticus* %
Ampicillin	0	15	0
Amikacin	25	33	50
Ceftazidime	15	0	0
Vancomycin	25	69	50
Piperacillin/ Tazobactam	50	50	100
Cefoperazone/Salbactem	50	10	50
Imepenum	25	69	100
Meropenum	25	15	50
Ciprofloxacin	50	41	50
linezolid	100	100	100

Gram negative bacteria were more common than gram-positive. Similar results are found by Muhammad *et al*, sheikh *et al*, Waseem *et al* and Basheer *et al*.[9, 11, 19, 20] *Coagulase Negative Staphylococcus* (CONS) and *Group B Streptococcus* are common Gram positive bacteria found in western countries but in our setup *Staphylococcus aureus* and *CONS* are common whereas *Group B streptococcus* is very uncommon.[8, 18, 21] Among Gram negative bacteria *E coli* is the commonest followed by *Pseudomonas, Klebsiella* and *Acinetobacter* but we found in our study *Pseudomonas* on top followed by *E coli*.[9, 11, 22]

Bacterial resistance to commonly used antibiotics in our settings such as Ampicillin and Cefotaxime was found quite high ranging between 85-100%. The study by sheikh *et al* found almost similar resistance pattern.[11] *Klebsiella* and *Proteus* showed 100% resistance to Amikacin while *Salmonella* and *Staphylococcuss aprophyticus* showed 50%. Arham *et al* found *Klebsiella* 40% sensitive to Amikacin and *Pseudomonas* 83% but in our study *Pseudomonas* was 33% resistant to Amikacin.[7] Resistance against quinolones, once found low compared to commonly used antibiotics, is emerging due to their indiscriminate use. Shaw *et al* found *Staphylococcus aureus, Streptococcus* and *Enterobacteriaceae* to be highly resistant to Ciprofloxacin,[20] and in our study resistance to Ciprofloxacin ranged between 35 to 90 % where *Enterobacter* is at 35% and *E.coli* at 90%. Imepenem is described by Waseem *et al* and Shaw *et al* as 100% sensitive to *Staphylococcus aureus, Acinetobacter, Klebsiella, E.coli* and *Enterobacteriaceae*[18, 20] but in our study *S. aureus* and *S. epidermidis* showed 75% and 69 % resistance to Imepenem while *Staphlococcus saprophyticus, klebsiella* and *Proteus* showed 100% sensitivity, Muhammad *et al* demonstrated high resistance of Imepenem against *Staphylococcus aureus*, but no resistance against *Acinetobacter, Klebsiella* and *Enterobacter*.[9] Linezolid found to be 100% sensitive to all gram positive isolates in our study and also by Arham *et al*, Li *et al* and Sheikh *et al*.[7, 8, 11]

Conclusion

The present study revealed that *Staph epidermidis* is the most common Gram positive bacterium and *Pseudomonas* is the most common Gram negative bacterium causing neonatal sepsis. All the bacterial isolates are highly resistant to commonly used antibiotics such as Ampicillin, Cefotaxime and Amikacin. Resistance is also emerging against Vancomycin and Ciprofloxacin. Gram positive organisms were highly sensitive to linzeolid.

Limitation of Study

Our study was conducted in a single neonatal unit so the information obtained about the pattern of microorganisms and their antibiotic sensitivities is specific to the unit and probably cannot be generalized to all the neonatal units in the city.

Moreover limited number of antibiotics was checked due to availability of limited kits.

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ORIGINAL ARTICLE

Clinical Characteristics and Risk Factors of Candidemia in Tertiary Care Hospital

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Abstract

Introduction
During the past two decades, the incidence of candidemia has doubled and *Candida* spp. currently ranks in top blood stream pathogen in developed countries. Given the need to ensure appropriate and timely antifungal therapy, there is need to identify these patients as early as possible and therefore a risk stratification for candidemia is imperative. We aim to identify the risk factors of candidemia in patients admitted at our tertiary care center.

Methods
A retrospective case control study were conducted on adult patients (15 years. or more) admitted to Aga Khan University Hospital between 2009 and 2013 who developed candidemia (cases)or bacteremia (controls) after 48 hours of admission.

Results
A total of 300 patients were enrolled in study (150 cases i.e. candidemia and 150 control i.e. bacteremia). The gender frequency was identical for cases (n=56, 65% males) and controls (n= 55,64 % males). Mean age in year was also similar for cases (56± 17) and controls (55± 18, p = 0.5). Non albican *candida* spp. are predominantly isolated from blood cultures as compared to *Candida albican*. Patients who had invasive devices like central lines, urinary catheter, endotracheal tube, nasogastric tube especially central lines (Odd ratio 1.72, CI: 0.98-3.02) and patients who had *candida* colonization (OR8.50, C.I: 3.76-19.23) were more likely to have candidemia than bacteremia.

Conclusion
Risk factors for candidemia include, the presence of invasive devices especially central lines and isolation of *candida* from other body sites were most predictive of candidemia. These results can be used to help identify patients most likely to benefit from empiric antifungal therapy.

Key words
candidemia, risk factors, *candida* colonization, invasive devices

Introduction
Candida species are one of the most frequent pathogens isolated in bloodstream infections[1] and are associated with significant morbidity and mortality. Blood stream infections (BSI) caused by various *Candida* species have been reported from many countries worldwide and are a significant cause of morbidity, prolonged hospital stay and mortality in hospitalized patients.[2,3,4,5,6] During the past two decades, the incidence of candidemia has been doubled and *candida* now currently ranks as the fourth and the seventh most common bloodstream pathogen in North American and Europe respectively[7,8]

The situation in Asia regarding the incidence of candidemia is however, not very clear due to lack of multicenter studies. A 13-year long study on candidemia from a tertiary care hospital in Thailand showed a prevalence of 6.14% for *Candida* species among blood culture isolates.[9] Similarly the prevalence of candidemia was between 6 % to 8% in different parts of India as reported by S Giri *et al*.[10] In Taiwan, candidemia was the second most common cause of health care-associated bloodstream infections (HA-BSIs), following after bacteremia by Staphylococcus species and *Acinetobacter baumannii* and *Escherichia coli*.[11]

Candidemia is often present in patient with underlying co morbidities and hence risk factors for candidemia include exposure to antibiotics, steroids, chemotherapy and total parental nutrition (TPN),or the presence of renal failure, neutropenia, invasive devices and candiduria.[3,9,10]

Unfortunately while delaying antifungal therapy in patients is often associated with poor outcomes such as prolonged hospital stay, morbidity and mortality,[6,12,13,14] the presentation of candidemia is non-specific and difficult to differentiate from bacteremia. This makes early identification of these patients paramount. While diagnostic biomarkers (β-D-glucan) have promise, blood cultures still remain the gold standard for diagnosis and take 24 to 72 hours for growth.

Given the need to ensure appropriate and timely antifungal therapy, there is need to identify these patients as early as possible. We therefore attempted to design a predictive model which would help differentiate bacteremia from candidemia early, allowing judicious initiation of early empiric anti-fungal therapy.

Materials and Methods
We conducted a retrospective case control study at a tertiary

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care teaching hospital in Karachi Pakistan. The Aga Khan University Hospital is a 700-bedded tertiary care referral hospital catering to a wide variety of patients from the region. The facility includes several ICUs and an active stem-cell and renal transplant service.

All patients above the age of 15 years who were admitted in hospital between January 2009 and December 2013 and had candidemia or bacteremia either on admission or during course of hospitalization were included in study. Cases with mixed blood stream infection and those with previous episodes of candidemia and bacteremia in last one month were excluded from study. Approval for the study was obtained from Human Ethics Review Committee, AKUH prior to initiation of data collection.

Medical records were reviewed and Information regarding patient demographics i.e. age and sex and risk factors like underlying co-morbid conditions (bone marrow transplantation, diabetes mellitus, chronic renal disease, chronic liver diseases) was collected as well as details regarding current treatment such as use of any antibiotics in previous 2 weeks, corticosteroids in last 30 days, chemotherapy in previous 30 days, presence of any invasive device (central venous pressure line(CVP), peripherally inserted central line(PICC line),urinary catheter, endotracheal tube), neutropenia, prior surgery within 30 days, *candida* isolated from any other site like urine, sputum , tracheal aspirate and administration of total parental nutrition at time of diagnosis were recorded.

Definitions

Candidemia was defined as the isolation of a *candida* species from at least one blood culture and bacteremia was defined as the isolation of pathogenic bacteria from at least one blood culture at time of admission or during stay in hospital. In cases where usual blood contaminants were isolated (e.g. *coagulase negative staphylococcus*, *corynebacterium* species), patients were included only if they had multiple culture positive for the same organism. Neutropenia was defined as a neutrophil count <500 neutrophils/mm. Invasive devices include central line (like central venous pressure line, peripherally inserted central line), urinary catheter, endotracheal tube, present at time of positive culture or removed within two days. *Candida* colonization is taken as isolation of *candida* species from body sites other than blood like urine, respiratory secretions, wound. Prior surgery within 30 days, chemotherapy within 30 days, use of antibiotics for last 15 days and steroids (any dose) for 30 days were considered as significant risk factors. Total parental nutrition at time of positive blood culture or within 48 hrs was considered significant.

Data Analysis

Descriptive analysis was carried out for demographic and clinical characteristics and results are presented as mean±SD for quantitative variables and numbers (percentages) for qualitative variables. To analyze the clinical characteristics of hospitalized patients with candidemia compared to bacteremia, the categorical variables were evaluated using the chisquare whereas the means were compared using the Student's t-test. All variables with p-value less than 0.25 on univariate analysis were then included in multivariable analysis. A stepwise procedure was used to select the variable with a value of $p<0.25$ as the inclusion criteria for best fitted multivariable model. P value less than 0.05 was considered to be statistically significant. The SPSS software(ver. 19) is used to perform all the statistical analysis.

Results

A total of 300 patients were enrolled in study (150 cases of candidemia and 150 with bacteremia). Demographic and clinical characteristics are shown in table1. Both groups were identical in gender (65% males with candidemia vs. 64 % males with bacteremia) and age (mean age of 56 +/- 17 vs. 55 +/- 18 for the candidemia and bacteremia group respectively).

Non albican *candida* species are predominantly isolated from blood cultures as compared to *Candida Albicans*. The distribution of bacterial and *candida* species among patients selected for this study is shown in Table. 2

Candida species were *Candida albican* (14%), *Candida parapsilosis* (14), *Candida tropicalis* (14%), *Candida glabrata* (5%) and *Candida krusei* (1%). Other *candida* species were *Candida lusitaniae* and *Candida guilliermondii*. Among bacterial species, *E. coli* was most commonly isolated in blood (30%). Other bacterial species isolated were *Acinetobacter* (8%), *Staphylococcus aureus* (17%), Coagulase-negative staphylococci (8%) and *Klebsiella pneumonia* (9 %). Bacterial species with low frequency were *Enterobacter*, *Proteus*, *Stenotrophomonasmaltophilia*, *Burkhoderiacepacia* and *Micrococcus.*

No clear risk factors were found differentiating candidemia from bacteremia such as presence of underlying medical illnesses, prior exposure to antibiotics, use of steroids, TPN, prior surgery and chemotherapy. However, patient with chronic liver disease were more likely to have bacteremia compared to Candidemia (OR = 0.43;95% CI: 0.21-0.88).

More patients with candidemia had invasive devices (p value 0.001). Among invasive devices, central lines were more common as compared to other devices (foley catheter , endotracheal tubes, nasogastric tube) in patients with Candidemia. More than half patients with Candidemia (60%) have two or more devices at time of positive culture.

Isolation of *candida* species from other body site culture like urine, sputum was also common in patients with Candidemia. Invasive device (OR = 1.72;95% CI: 0.98-3.02) and *candida* isolated from other body site like urinary (OR = 8.50;95% CI:

Table 1: Demographic and clinical characteristics of hospitalized patients with candidemia and bacteremia.

	Bacteremia (n=150)(%)	Candidemia (n=150)(%)
Age(years)	54.8 ± 18.2	56.05 ± 16.9
Gender		
Male	92(63.9)	95(65.1)
Female	52(36.1)	51(34.9)
Type of Malignancy		
Solid organ	25(61)	26(55.3)
Hematological	16(39)	21(44.7)
Diabetes Mellitus	47(31.5)	55(37.7)
Chronic liver disease	31(20.8)	19(12.8)
Chronic kidney disease	22(14.8)	29(19.3)
Bone marrow transplant	0	1(0.7)
Hemodialysis	20(13.4)	17(11.3)
Previous Antibioticstherapy[a]	76(52.8)	100(66.7)
Steroids use [b]	42(29.2)	38(25.3)
Chemotherapy[c]	23(16)	21(14)
Invasive devices[d]	91(62.8)	120(80)
Neutropenia[e]	13(9.6)	20(13.3)
Total parental nutrition[f]	19(13.2)	15(10)
Prior surgery[g]	45(31.7)	41(27.3)
Candida species isolated from other body site[h]	8(5.8)	51(34)

a. Exposure to antibiotics for last 15 days , b. Patient receiving steroids for last 30 days c. Chemotherapy for last 30 days ,d .Presence of invasive devices like central venous line , foley's catheter, endotracheal tube, e . Neutrophils count <500 neutrophils/mm, f. Total parental nutrition at time of diagnosis, g. Any surgery within 30 days , h. isolation of *candida* from other body site like urine, sputum, tracheal aspirates , wounds

3.76-19.23) were found to be stronger predictors for candidemia. Refer to Table 3 and Table 4.

Table 2: Microbiological spectrum in patients with candidemia and bacteremia

Microorganisms	Candidemia n(%)
Candida Albican	42(14)
Candida parapsilosis	43(14.3)
Candida tropicalis	41(13.6)
Candida glabrata	15(5)
Candida krusei	3(1)
Candida lusitaniae	2(0.7)
Candida guilliermondii	2(0.7)
Microorganisms	**Bacteremia n(%)**
E coli	53(29.7)
Staphylococcus aureus	30(16.8)
Coagulase-negative staphylococci	5(8.4)
Acinetobacterspecies	15(8.4)
Klebsiellapneumoniae	16(8.9)
Enterococcus	10(5.6)
Pseudomonas aeruginosa	7(4.6)
Streptococcus group D	8(5.3)
other bacteria[a]	23(15.3)

a. Other bacteria included Enterobacter, Proteus, Stenotrophomonas maltophilia, Burkhoderia cepacia and Micrococcus

Discussion

Our study found the presence of invasive devices like central lines and isolation of *candida* species from body sites other than blood were significant risk factors for developing candidemia and there was a predominance of non albican *candida* species isolated from the blood**.**

Among all invasive devices, presence of central venous catheter was found to be significant risk factor for candidemia in our study. This is in accordance with previous studies which also identify central catheter as an important predisposing factor for invasive candidiasis.[3,10,15,16,17,18]

Candida is known to form biofilm on medical devices which provide a nidus for infection and presence of biofilm make *candida* species relatively resistant for antifungal therapy as well. Removal of invasive device is sometimes necessary for management of *candida* blood stream infection.

Majority of candidemic patients in current study also had *candida* isolated from other body sites especially urine. This confirms the relationship between colonization by *candida* species (particularly multifocal colonization) and the increased risk of invasive *candida* infection.[19,20,21,22] A study from Turkey also found candiduria as an independent risk factor for candidemia.[23] *Candida* species are part of endogenous flora colonize mucocutaneous surfaces. *Candida* colonization is one of pre-requisites for invasive candidiasis, which in the presence

Table 3: univariate analysis of risk factors for candidemia

	Bacteremia n=150	Candidemia n=150	Odd ratio[95% CI]	p value
Type of Malignancy				
Hematological	16(39)	21(44.7)	1.0	
Solid organ	25(61)	26(55.3)	0.79[0.33-1.85]	0.59
Diabetes Mellitus				
No	102(68.5)	91(62.3)	1.0	
Yes	47(31.5)	55(37.7)	1.31[0.81-2.12]	0.26
Chronic liver disease				
No	118(79.2)	130(87.2)	1.0	
Yes	31(20.8)	19(12.8)	1.79[0.96-3.35]	0.06
Chronic kidney disease				
No	127(85.2)	121(80.7)	1.0	
Yes	22(14.8)	29(19.3)	1.38[0.75-2.54]	0.29
Hemodialysis				
No	129(86.6)	133(88.7)	1.0	
Yes	20(13.4)	17(11.3)	0.82[0.41-1.64]	0.58
Antibiotics[a]				
No	68(47.2)	50(33.3)	1.0	
Yes	76(52.8)	100(66.7)	1.78[1.11-2.86]	0.01
Steroids[b]				
No	102(70.8)	112(74.7)	1.0	
Yes	42(29.2)	38(25.3)	0.82[0.49-1.37]	0.46
Chemotherapy[c]				
No	121(84)	129(86)	1.0	
Yes	23(16)	21(14)	0.85[0.45-1.62]	0.63
Invasive device[d]				
No	54(37.2)	30(20)	1.0	
Yes	91(62.8)	120(80)	2.37[1.40-4.00]	0.001
Neutropenia[e]				
No	123(90.4)	130(86.7)	1.0	
Yes	13(9.6)	20(13.3)	1.45[0.69-3.05]	0.32
Total parental nutrition[f]				
No	125(86.8)	135(90)	1.0	
Yes	19(13.2)	15(10)	0.73[0.35-1.50]	0.39
Prior surgery[g]				
No	97(68.3)	109(72.7)	1.0	
Yes	45(31.7)	41(27.3)	0.81[0.49-1.34]	0.41
Candida isolated from other body site[h]				
No	130(94.2)	99(66)	1.0	
Yes	8(5.8)	51(34)	8.37[3.80-18.44]	<0.001

a. Exposure to antibiotics for last 15 days, b. Patient receiving any steroids for last 30 days, c. Chemotherapy for last 30 days, d. Presence of invasive devices like central venous line , foley's catheter, endotracheal tube at time of diagnosis, e. Neutrophils count <500 neutrophils/mm, f. Total parental nutrition at time of diagnosis, g. Any surgery within 30 days, h. isolation of *candida* from other body site like urine, sputum, tracheal aspirates, wounds.

Table 4: Multivariate logistic regression analysis of risk factors for development of Candidemia

	Odds ratio[95% CI]	p value
Chronic liver disease		
No	1.0	
Yes	0.43[0.21-0.88]	0.02
Candida isolated from other body site		
No	1.0	
Yes	8.50[3.76-19.23]	<0.001
Invasive devices		
No	1.0	
Yes	1.72[0.98-3.02]	0.056

Presence of invasive devices like central venous line, foley's catheter, endotracheal tube at time of diagnosis

of certain risk factors like immunosuppressive state, neutropenia, surgery, antacid therapy, invasive devices, prolonged antibiotic use, can lead to invasion of muco -cutaneous barrier and haematogenous spread. *Candida* colonization is therefore an important factor to consider for empirical antifungal therapy in high risk population.[24,19]

Our study also found a predominance of non albican *candida* species of which *Candida parapsilosis* and *Candida tropicalis* were most common species isolated. Old age, severe immunosuppression or illness, prior antifungal therapy, exposure to broad spectrum antibacterial agents and presence of central lines contribute to increased incidence of candidemia caused by non albican species of *candida*.[1,16] Majority of our candidemic patients were older than 50 years of age, had malignancy and DM, prior exposure to antibiotics and invasive devices, which explain the preponderance of non albican *candida* species in current study. *Candida parapsilosis* is found on skin surfaces and has ability to form biofilms on catheter and other implanted devices. It can easily be spread by hand carriage and it persists in hospital environment. *Candida* parapsilosis has been increasingly implemented as a cause of candidemia after placement of venous catheter. As most of our patient has central lines, this can be the possible reasons of *candida* parapsilosis being isolated as dominant species.

The distribution of *candida* species causing candidemia is different in different geographical areas. *C.albicans* is predominant species in North and central Europe and the USA. On the contrary non albicans *candida* species is more frequently isolated in Asia , South Europe and South America.[25]

Data from India also has relatively higher incidence of non albican *candida* species with higher frequency of *C. tropicalis* and *C. parapsilosis*. A recent study from Middle East also found predominance of non albican Candidemia but species distribution is different. It found high proportion of *C. glabrata* followed by *C. tropicalis* and *C. parapsilosis*.[26] Data from Pakistan on invasive candidiasis and candidemia is limited. Other studies from Pakistan also found non albican *candida* species to be more common in patients with invasive candidiasis with a high prevalence of *C. tropicalis* and *C. parapsilosis*.

Most common risk factors for candidemia like DM, neutropenia, prior exposure to antibiotic and chemotherapy, prolonged use of steroid were not able to reach statistical significance between two groups in our studies. This study was done in single institutes and had small sample size, which could be the possible reason of above findings. There is a need of multi centre studies to identify the risk factors for candidemia.

Conclusion

Presence of invasive devices and isolation of *candida* from different body sites were important independent risk factors for candidemia and can be consider to start early empirical antifungal therapy in suspected cases of blood stream infections.

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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 into 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.

IV. Case Reports
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:

- Black& white line illustration (e.g. graphs): 600 dpi
- Black & white halftone illustrations (e.g. photographs): 300 dpi
- Color illustrations: 400 dpi (note that color images should be split CMYK not RGB)

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.

Instructions updated - April 2012.
Editor IDJ