A Microbiological Study of Bacterial and Fungal Etiology in Various Clinical Specimens of Patients in Dawadimi General Hospital

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Abstract: Objectives: To detect bacterial and fungal organismson clinical specimens which were collected from hospitalized and out -door patients inDawadimi GeneralHospitals. **Material and methods**:This study was carried out on various clinical specimens of patients in Dawadimi General Hospitals. All clinical samples except urine samples were collected aseptically and inoculated on plates of Blood agar, MacConkey agar, Sabouraud Dextrose agar and Nutrient agar, whereas urine samples were inoculated on plates of CLED agar ,Sabouraud Dextrose agar and Nutrient agar (Oxoid Cambridge, UK). This was followed by identification of bacteria and fungi. **Results**: 60 nosocomial isolates (86.95%) and 74 out-door isolates (132.1%) were collected.*Pseudomonas aeruginosa* showed maximum percentage incidence in both of community acquired infections (17.56%) and nosocomial infections (16.66%), followed by *Escherichia coli,* (14.86%) in community acquired infections and *Staphylococcus aureus* (13.33 %) in nosocomial infections.The most prevalent fungal organisms.in both out- door and hospitalized patients were*Candida spp.* with frequencies 36.48% and 31.66%, respectively.
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Key words: Clinical specimens, Nosocomial infections, Community acquired infections, *Pseudomonas aeruginosa*, *Escherichia coli*, *Candida spp*.

1. Introduction

Infectious diseases remain a major cause of debility and death around the world and are responsible for worsening of living conditions of millions of people(Hossein*et al*., 2010). Microbes (bacteria, fungi, parasites and viruses) cause infectious diseases and antimicrobial agents have been developed to combat the severity and spread

of many of these diseases. The microbial infections are classifiedinto two essential categories, nosocomial infections and communityacquired infections.An infection

is considered as nosocomial infection by center for disease controlif it occurs due to exposure to hospital environment while according to Qayyum*et al*.(2010) if an infection is caused at least after 48 hours of admitting a patient in a hospital is a nosocomial infection (Sheikh *et al*.,2008;Qayyum*etal*.,2010). Along with the patients already admitted in hospital, healthy person may be also affected who are in regular contact of hospital's environment (Qayyum*et al*.,2010).Nosocomial infections was first reported in 1980s in Europe caused by bacteria especially enterobacteriaceae which were producing extended spectrum β-lactamase(Jain *et al*., 2003).Community acquired infections, can be defined as onset of infections prior to hospital admission and not within 10 days of hospital discharge(Maya *et al*.,2010).The use of antimicrobial agents for prevention or treatment of infections in humans in any dose and over any time period, cause a "selective pressure" on microbial populations(Sharafati*et al*., 2010).The emergence of resistance to antimicrobial in previously susceptible bacterial and pathogens is a major challenge to infectious disease medicine.(Ahsan *et al*.,2011).

Different bacterial genera *Escherichia*, *Klebsiella*, *Enterobacter*, *Serratia,Citrobacter* and *Proteus* include obligatory and opportunistic pathogens responsible for a wide range of infections. Many species are members of the normal intestinal flora. *Escherichia coli* is the most commonly isolated organism in the clinical laboratory(Guentzel,1995).*Klebsiella*, *Enterobacter*, and *Serratia* species are frequent causes of bacteremia and also are frequently involved in infections associated with respiratory tract manipulations, such as tracheostomy and procedures using contaminated inhalation therapy equipment. *Proteus*, *Klebsiella* and *Enterobacter* species are among the other organisms most frequently involved in urinary tract infections following intravenous and urinary catheterization and infections complicating burns. *Proteus* species frequently cause nosocomial infections of the urinary tract, surgical wounds, and lower respiratory tract. *P. mirabilis*, is believed to be the most common cause of infection-related kidney stones, one of the most serious complications of unresolved or recurrent bacteriuria(Ali *etal*., 1998).*Escherichia coli* causes approximately 85 percent of cases of urethrocystitis,

about 80 percent of cases of chronic bacterial prostatitis, and up to 90 percent of cases of acute pyelonephritis (Guentzel,1995).Bacterial pneumonia accounts for 15% of all hospital-associated infections and is the second most common hospital-acquired infection (urinary tract infections remain the most common nosocomial infection). *Pseudomonas aeruginosa*, *Enterobacter*, *Klebsiella pneumoniae*, *Escherichia coli*, *Serratiamarcescens* and *Proteus* species are the most frequently isolated pathogens causing nosocomial pneumonias infections. (Shah*et al*., 2002).

Nosocomial blood stream infections are usually caused by gram-positiveorganisms including ,*Staphylococcus auerus* and *Enterococci* (Nosocomial infection, 2009). Although gram-negative organisms historically accounted for most cases of nosocomial pneumonia, the proportion of nosocomial infections due to gram-positive organisms has increased over the past 15 years. The most important gram-positive pathogen of nosocomial respiratory tract infections is *S. aureus*(Richards *etal*., 1999).

In case of community acquired infections, *E. coli* is the major cause of urinarytract infections, including prostatitis and pyelonephritis.*Proteus*, *Klebsiella* and *Enterobacter* species are also common urinary tract pathogens, *Klebsiella pneumoniae* accounts for a small percentage of pneumonia cases, however,extensive damage produced by the organism results in high case fatality rates (upto90 percent in untreated patients))Guentzel, 1995).

In recent years, yeasts (ex: *Candida albicans, Cryptococcus neoformans, Cryptosporidium sp., Malassezia spp.,* and *Saccharomyces cerevisiae*)and molds(ex: *Aspergillus spp.*) have emerged as important pathogens(Arunaloke*et al.*,2008).These fungi area leading cause of morbidity and mortality in cancer, burn, and surgical patients as well as neonatal intensive care unit patients(Crump *et al*. ,2011). Advances in newer technologies in medical and surgical therapies, use of invasive monitoring devices and broad-spectrum antimicrobial agents over the past twodecades have helped to treat patients suffering from previously devastating or fatal diseases, but they have however, changed the type of patients cared for in our hospitals. These successes have resulted in proliferation of many severely ill and immunocompromised individuals who are highly susceptible to infections caused by fungi that were previously considered to be of low virulence.Consequently, infections due to previously obscure fungi are being seen more commonly in hospitalized patients. Fungal infections in these patients are often severe, rapidly progressive, and difficult to diagnose or treat. Early initiation of antifungal therapy iscritical in reducing the high mortality rate in these patients(Bineeta*etal*., 2012).

The aim of the present study was to determine the percentage incidence of bacteria and fungi according to hospital and community acquired infections, ward, specimens and gender of patients**.**

2. Material and Methods

Study design:

The present work is a descriptive cross- sectional study which wascomprised clinical samples received from 125 patients through period of 3 months (October 2013 to December 2013) in the Microbiology Laboratory of Al-Dawadimi General Hospital in Saudi Arabia.

The Microbiological analysis was carried out at Microbiology Laboratory in Applied Medical Science Collage to find out the incidence of bacteria and fungi in nosocomial and community acquired infections.

Specimens collection:

Fifty six clinical specimens were collected from the out- door patients while the other 69clinical specimens were collected from patients hospitalized in different wards (Post-operative surgical and Intensive care unit) of Al-Dawadimi General Hospitals,Dawadimi, Saudi Arabia.The 125 clinical samples include 15 blood samples,47 urine samples and sixty three different swabs. Different clinical swabs such as HVS (14), throat (9), nasal (9), axilla (6), wound (8), groin (7), nail (5), pus (3), urethral (1)and one eye swab.

Cultivation and Identification:

All clinical samples except urine samples were aseptically inoculated on plates of Blood agar, MacConkey agar,Sabouraud Dextrose agar and Nutrient agar, whereas urine samples were inoculated on plates of Cystine-Lactose-Electrolyte-Deficient (CLED) agar ,Sabouraud Dextrose agar and Nutrient agar (Oxoid Cambridge, UK).All inoculated plates except Sabouraud Dextrose agar plates were incubated at 37ºC for 24 h. The inoculated plates of Sabouraud Dextrose agar were incubated at two degrees 28 ºC for 5 days and 37ºC for 48h.The identification of bacteria was done by macro and micromorphological evaluation. The characteristics considered were size, shape, colour, pigmentation and haemolyticnature of colonies .Also biochemical tests were applied by using conventional methodsand API. (20NE).The identification of fungi was also done by macro and micro morphological evaluation. Micro culture on slides was the technique used for observation of filamentous fungi. The yeast isolates were identified by Chromogenic agar medium.

3. Results

Sixty nine clinical samples were collected from hospitalized patients (48 men, 19 women and 2 child). These samples include (15)blood,

(12)urine,(9)nasal,(8)wound,(6) axilla, (7) throat, (7)groin and five nails.Sixty nosocomial isolates (86.95%) which include bacteria(38 isolates, 63.33%) and fungi (22 isolates, 36.66%) were collected. Fourteen of these isolates(8 bacterial isolates and 6 fungal isolates) were isolated from urine,5 isolates (4 bacterial isolates and 1 fungal isolates), from wound swabs, 11 isolates (7 bacterial isolates and 4 fungal isolates) from throat swabs,8 isolates (5 bacterial isolates and 3 fungal isolates) from nasal swabs,6isolates (5 bacterial isolates and 1 fungal isolate) from blood,5 isolates (2 bacterial isolates and 3 fungal isolate) from nail swabs, 10 isolates(6 bacterial isolates and 4 fungal isolates) from groin swabs and one bacterial isolate from axilla swabs.Out of these isolates the most prevalent bacterial organism was *Pseudomonas aeruginosa* 10 (16.66%) followed by *Staphylococcus aureus* 8 (13.33 %),*Streptococcus agalactiae*3(5%), *Streptococcus pneumoniae* 3 (5%), *Escherichia coli* 3(5%), *Streptococci viridanis* 2 (3.33%) and *Klebseilla pneumoniae* 2 (3.33%). The percentage incidence of the remain bacterial organisms was 1.66%.Among these 10 isolates *Pseudomonas aeruginosa* was most prevalent in urine, 3 out of 10 (30 %), 2 from both groin and wound swabs (20%) and one from nasal, throat and nail swabs (10%).

The most prevalent fungal organisms were *Candida spp.* 19 (31.66 %). *Candida albicans* showed high percentage incidence (18.33%) flowed by *Candida krusei*(13.33%). The percentage incidence of *Drechslera sp.*, *Rhodotorula sp.* and *Penicillium sp.* was very low (1.66%).as shown in Table 1&2.

Table (1): Distribution of nosocomial bacterial isolates

Clinical specimens	*P. aeruginosa*	*S. aureus*	*S. agalactiae*	*S. pneumoniae*	*E. coli*	*S. viridans*	*Klebsiellapn eumoniae*	*Staph. epidermidis*
Urine	3	-	-	-	2	-	-	-
Throat wabs	1	1	1	1	-	2	1	-
Blood	-	2	-	-	-	-	-	1
Groin swabs	2	1	1	-	1	-	-	-
Nasal swabs	1	2	-	2	-	-	-	-
wound swabs	2	1	1	-	-	-	-	-
Nail swab	1	-	-	-	-	-	1	-
Axilla swabs	-	1	-	-	-	-	-	-
Total	10	8	3	3	3	2	2	1

Table (1): Continued.

Clinical specimens	*Acinetobacter baumannii*	*Neisseria sp.*	*Streptococcus faecalis*	*Proteus sp.*	*Staph. mominis*	*Serratia marcescens*	*Total*
Urine	1	1	1	-	-	-	8
Throat swabs	-	-	-	-	-	-	7
Blood	-	-	-	1	1	-	5
Groin swabs	-	-	-	-	-	1	6
Nasal swabs	-	-	-	-	-	-	5
Wound swabs	-	-	-	-	-	-	4
Nailswabs	-	-	-	-	-	-	2
Axilla swabs	-	-	-	-	-	-	1
Total	1	1	1	1	1	1	38

Table (2):Distribution of nosocomial fungal isolates.

Clinical specimens	*Candida albicans*	*Candida krusei*	*Drechslera sp.*	*Penicillium sp.*	*Rhodotorula sp.*	*Total*
Urine	3	2	-	1	-	6
Throat swabs	3	1	-	-	-	4
Blood	1	-	-	-	-	1
Groin swabs	1	2	-	-	1	4
Nasal swabs	2	1	-	-	-	3
Wound swabs	1	-	-	-	-	1
Nail swabs	-	2	1	-	-	3
Axilla swab	-	-	-	-	-	-
Total	11	8	1	1	1	22

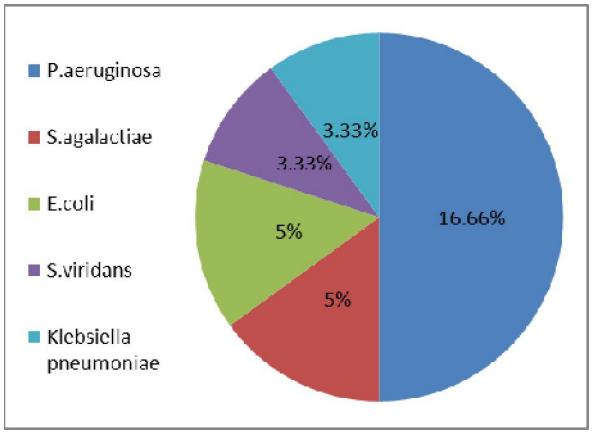

Fig.1- The percentage incidence of most common nosocomial bacterial isolates in clinical specimens collected from hospitalized patients.

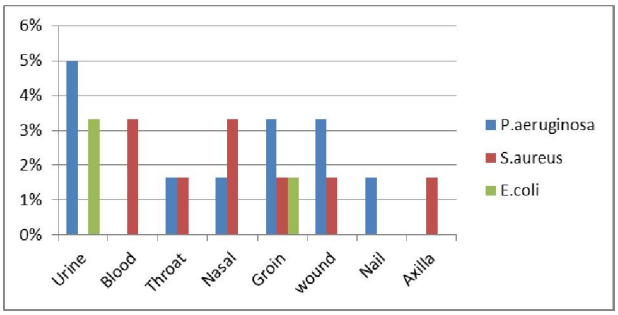

Fig 2- Percentage incidence of most common nosocomial bacterial species collected from different types of clinical specimens collected from hospitalized patients.

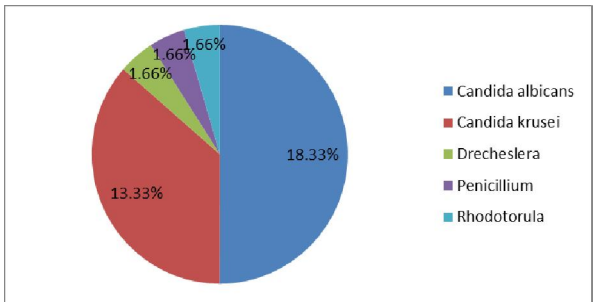

Fig3- The percentage incidence of most common nosocomial fungal isolates in clinical specimens collected from hospitalized patients.

Fifty six clinical samples collected from out-patient department(forty six women and 10 men) during the study period. These samples include (35) urine, (14) HVS swabs,(3)pus swabs,(2)throat swabs, (1) urethral and one eye swab.Seventy four out- door isolates (132.1%) which include bacteria(44 isolates 78.57%) and fungi (30 isolates 53.75%) were isolated from clinical samples.Fifty one of these isolates(30 bacterial isolates and 21 fungal isolates) were isolated from urine,16 isolates (9bacterial isolates and 7 fungal isolates), from HVS swabs, 5isolates (3bacterial isolates and 2 fungal isolates) from pus swabs,1 bacterial isolate from urethral swab and onebacterial isolate from eye swab.Among these isolates *Pesudomonas aeruginosa* was the most abundant, 13 out of 74 (17.56%) followed by *Escherichia coli,*11 out of 74 (14.86%). Six isolates of*Staphylococcus aureus* (8.10%), 5 *Neisseria sp.* (6.75%), 4 *Klebsiella pneumoneae* (5.40%), 3*Streptococcus viridians* (4%) , 1 *Proteus vulgaris* (1.35%) and also 1 out of 74 (1.35%) isolate of *Serratia marcescens* was obtained from out- door isolates. Among these 13 isolates*Pseudomonas aeruginosa* was most prevalent in urine, 12 out of 13 (92.30%) and 1 (7.6%) from eye. Among these 11 isolates *Escherichia coli* was most prevalent in urine, 6 out of 11 (54.54%), 4from HVS (36.36%) and 1 from pus (9%).*Staphylococcus aureus* was most prevalent in urine, 3 out of 6(50%) and one from HVS, pus and urethral swaps (16.66%).*Neisseria sp.*was most prevalent in urine only,5 out of 5 (100%). Among these 4 isolates, *Klebsiella pneumoneae*was most prevalent in urine 3 out of 4(75%) and only one isolate from HVS swab (25%). *Streptococcus viridans* was found in HVS swabs, 2 out of 3(66.6%) and one from pus swabs (33.33%).

The most common fungal isolates from out – door patients were *Candida spp.* with high percentage incidence (36.48%).whereas, the incidence of *Candida albicans* was (24.32%) and *Candida krusei* was(12.16%).

Table(3):Distribution of out- door bacterial isolates.

Clinical specimens	*P. aeruginosa*	*S. aureus*	*Neisseria sp.*	*Proteus vulgaris*	*E. coli*	*S. viridans*	*Klebseillapneumoniae*	*Serratia marcescens*	Total
Urine	12	3	5	-	6	-	3	1	30
HVS swabs	-	1	-	1	4	2	1	-	9
Pus swabs	-	1	-		1	1	-	-	3
Urethral swabs	-	1	-	-	-	-	-	-	1
Eye swabs	1		-	-	-	-	-	-	1
Total	13	6	5	1	11	3	4	1	44

Table(4):Distribution of out- door fungal isolates

Clinical specimens	*Candida albicans*	*Candida krusei*	*Geotrichum*	*Rhodotorula*	Total
Urine	18		2	1	21
HVS swabs		7	-	-	7
Pus swabs	-	2	-	-	2
Urethral swabs	-	-	-	-	-
Eye swabs	-	-	-	-	-
Total	18	9	2	1	30

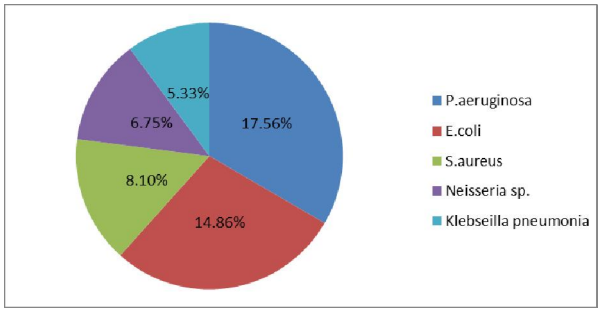

Fig4- **The percentage incidence of most common bacterial isolates in clinical specimens collected from out-door patients.**

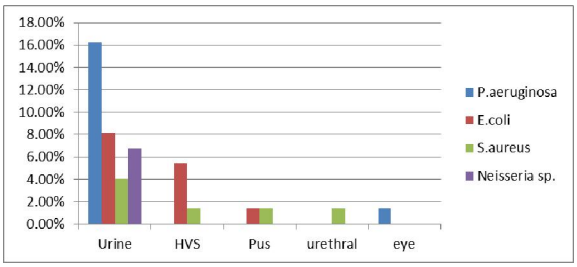

Fig 5- **Percentage incidence of most common bacterial species collected from different types of clinical specimens collected from out -door patients.**

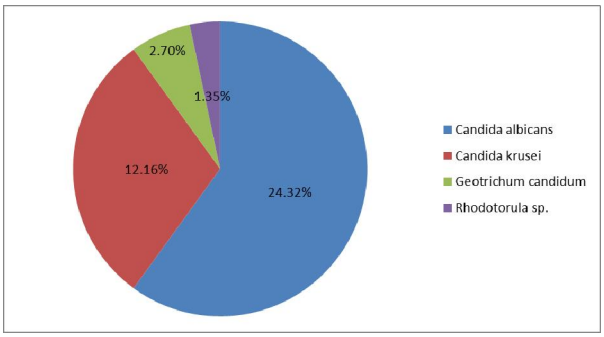

Fig6- **The percentage incidence of most common fungal isolates in clinical specimens collected from out – door patients.**

4. Discussion

The results of the present studyrevealed that the rate of community acquired infections higher than the rate of nosocomial infections.*Pseudomonas aeruginosa* showed maximum percentage incidence in both of community acquired infections (17.56%) and nosocomial infections (16.66%), followed by *Escherichia coli,* (14.86%)and *Staphylococcus aureus* (13.33 %) in case of nosocomial infections.The percentage incidence of *Neisseria sp.* was 6.75%, followed by *Klebsiella pneumonia* 5.40% in out - door *patients* whereas their incidenceinhospitalized patients were 1.66%.

Streptococcus agalactiae, *S.pneumoniae*, *S. viridans,Staph. epidermidis*, *Acinetobacter baumannii*, *Streptococcus faecalis*, *proteus sp*., *staph. Mominis* and *Serratiamar cescens.* Were isolated from hospitalized patients with low frequencies ranged from (1.66%-5%). Also *Streptococcus viridians*, *Proteus vulgaris* and *Serratia marcescens* were isolated in low frequencies ranged from (1.66%-5%) in specimens of out- door patients. Gram-negative bacteria were more significantly involved in infections than Gram- positive bacteria and the high percentage incidence of isolates 30.4% were from urine in both of nosocomial and community acquired infections. *Pseudomonas aeruginosa* and *Escherichia coli* were the most prevalent etiological agents in urine samples, *Staphylococcus aureus* was the most prevalent pathogen in blood and nasal samples.

This predominance of Gram-negative bacteria was coincident with the finding of some recent studies, Shalini *et al*., 2010 isolated seventy one bacterial isolates, 63 gram negative and 8 gram positive bacteria from different clinical samples of out-door patients. The high incidence of isolates 60.5% were from urine samples, while 9.7, 8.4, 5.6, 1.4% were from stool, pus, high vaginal swab, blood culture and sputum respectively. *Escherichia coli* (54.9%) was the most prevalence bacterial pathogen in various sample, followed by *Klebsiella pneumonia* 15(21.1%), *Pseudomonas aeruginosa* 8 (11.2%), *Enterococcus fecalis* 8(11.2%) and *Proteus mirabilis* 1(1.4%).

(Abdel-Fattah, 2005) found that from a total of 1382 patients had developed infection during hospital admission, 668 (48.3%) had nosocomial infection and 714 (51.7%) had community-acquired infection. Gram-negative organisms were reported in 66.2%, *E. coli* was the commonest (22.3%), followed by *Pseudomonas aeruginosa* (17.6%) and *Klebsiellapneumoniae (*9.9%). *Acinetobacter spp.* while Gram-positive organisms were reported in 31.8%. MRSA (Methicillinresistant *S. aureus*) was the commonest (10.2%), followed by

coagulase negative staphylococci (8.5%) and MSSA (Methicillin-susceptible *S. aureus*, 7.4%).While Shahidullah *et al.*, 2012 found that the most common bacterial pathogen in different clinical specimens which include blood, urine, pus, pericardial fluid, swab from wound, conjunctiva and throat was *Pseudomonas* species 39(14.2%) followed by *Escherichia coli* 37(13.5%), *Staphylococcus saprophyticus* 19 (6.9%) *and Staphylococcus aureus* 6 (2.2%). *Pseudomonas* species was isolated mostly from pus which was 29 (31.5%) cases. *E. coli* was found most commonly in pus and urine as well which were 15(16.3%) cases and 14(14.1%) cases respectively. *Staph saprophyticus* and *Staphylococcus aureus* were both found most commonly in pus which was 12(13.0%) cases and 4(4.3%) casesrespectively.

Nicolletti*et al.*, 2006;Hassanzadeh *et al.*, 2009 suggested that the predominance of Gram- negative bacteria may be due totheir wide prevalence in the hospital environment, and most of them represented a widerange of normal flora. In addition, their frequent resistance to antibiotic may play a role intheir persistence and spread.

In our study *Enterobacteriaceae* represented the most frequently isolated pathogens in a similarityto these results recorded by Jawady and Al-Habib, 2012 who isolated different bacterial isolates from urine, respiratory and wound samples. He found that the predominant isolate for Gram-negative bacteria was *E.coli* 16(23.2%), followed by *Pseudomonas spp*. 15(21.7%), where *P.aeruginosa*comprised 10 (66.7%), *K.pneumoniae* 10(14.5%), *Proteus* spp. 8(11.6%), where *P.mirabilis* constituted 6(75%) and *P.vulgaris* 2(25%), *Acinetobacter*spp. 6(8.7%), *E.aerogenes*2(2.9%). On the other hand, the predominant Gram-positive bacterial isolate was *S.aureus* 5(7.2%), followed by *E.faecalis* 4 (5.8%), and *S.epidermidis*3(4.3%).

Bennett, *et al* (1995) concluded that *E. coli*, *Pseudomonas* species, *Klebsiella* species and *Enterococcus* have been the predominant microorganisms that cause UTIs in patients withSCI. A high prevalence of *Proteus* species, which may relate to the more frequent use of indwelling catheters, has been noted at some centers.

This study revealed that the most common fungal organisms were *Candida spp.* in both of out-door and hospitalized patients with frequency 36.48% and 31.66%, respectively.Kashyap*et al.*(2012) found that the most common fungal pathogen was *Candida spp*. 36(66.6%),followed by *Aspergillus spp*. 17(31.4%) during their studies on 198 clinical samples which were obtained from the hospitalized patients and their places. Candida species isolated from the cases that was proved with a Candida infection and included: *C. albicans, C. tropicalis, C .glabrata, C. krusei, C.guilliermondii and C. parapsilosis*.

The majority of nosocomial fungal infections were reported tobe caused by *Candida* spp.At hospitals reporting data to the NNIS system during 1980 to 1990, *Candida* infections accounted for 78.3% of nosocomial fungal infections,followed by *Candida glabrata* (7.3%) and *Aspergillus spp.* (1.3%) (8).Many institutions have reported newly recognized pathogenic fungi, previously thought to be nonpathogenic, including *Malasezzia*spp., non-*albicans Candida* spp., *Fusarium* spp., and *Trichosporon*spp. (Samuel *et al.*, 2010).

Finally our recorded results is nearly close to these results recorded by Pathan*et al.*(2013)whereas, among total 508 specimens were involved, 32 (6.30%) clinical specimens were positive for the presence of fungi while remaining 476 specimens were negative for the presence of fungi. In these 476 specimens, 88 specimens indicated no growth of organisms while remaining 388 specimens showed the growth of pathogenic bacteria. In these 388 specimens, 85 isolates were Gram positive bacteria and 303 isolates were Gram negative bacteria. The Gram positive bacterial species isolated and identified were *Staphylococcus spp., Enterococcus spp.* and *Streptococcus spp.* The Gram negative bacteria isolated and identified were *Pseudomonas spp., Escherichia spp., Klebsiella spp., Morgenalla spp.* and *Salmonella spp.* Out of these 32 fungal positive patients, 16 were from Outdoor patient & remaining 16 were from various wards (Indoor department). The study indicated that the fungi to be isolated were *Candida spp.* from the sputum, nail, stool, urine and body fluid. The most predominant species was *Candida albicans*14/32 (43.75%), followed by *Non albicans Candida* species 11/32 (34.37%).

Conclusion:

Infectious diseases remain a major cause of debility and death around the world and are responsible for worsening of living conditions of millions of people. The rate of community acquired infections higher than the rate of nosocomial infections. Gram-negative bacteria were more significantly involved in infections than Gram- positive bacteria and the high percentage incidence of isolates were from urine in both of nosocomial and community acquired infections *Pseudomonas aeruginosa* showed maximum percentage incidence in both of community acquired infections and nosocomial infections. The most common fungal organisms were *Candida spp*. in both of out- door and hospitalized patients.So it is necessary to diagnosis the bacteria and fungi as etiological agents in nosocomial and community acquired infections .

Early detection of bacteria and fungi helps clinician in description of antibacterial &antifungal drugs .

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