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### RESEARCH ARTICLE

## STUDY ON THE BACTERIAL ISOLATES FROM HAND SWAB SAMPLES OF HEALTH CARE WORKERS AND ANTI-BIOTIC SENSITIVITY PATTERN , IN A TERTIARY CARE HOSPITAL , LUCKNOW

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#### Manuscript Info

#### Abstract

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#### Introduction:-

The contaminated hands of health care workers (HCW), play an important role in transmission of hospital infection, and is a common pattern seeing in most healthcare settings[1]. An important cause of morbidity and mortality among hospitalized patients worldwide, is Healthcare-associated infections (HCAIs), also known as nosocomial infections[2]. In developed countries, HCAIs have been reported to affect 5%-15% of hospitalized patients and 9%-37% of those admitted to intensive care units (ICUs), while in developing countries , prevalence rates have been estimated to be between 14.8% and 19.1%[3]. The single most important measure to prevent cross-transmission of microorganisms and to reduce the rate of nosocomial infection is to maintain hand hygiene[4]. The landmark study carried out by Semmelweis in 1884 , demonstrated that the simple act of hand washing could save lives especially when health care workers do it routinely and thoroughly[5]. The organisms transmitted can be harmful for both patients and health-care workers ,and these organisms are sometimes resistant to antimicrobial agents (AMA) .Still, health-care workers(HCW), do not practice hand washing[6].

To reduce such infections in hospital, a targeted surveillance culture to identify asymptomatic carriers of multidrug resistant bacteria and subsequent isolation and treatment should be done. To predict possible risk of infection with multi-drug resistant bacteria in health-care settings , the sensitivity patterns of isolates should also be studied.

This study was therefore undertaken in a TERTIARY CARE HOSPITAL , LUCKNOW as part of a wide scale hospital infection control surveillance programme. It seeks to ascertain the proportion of bacterial colonization of the isolates from the hands of healthcare workers who are in regular contact with patients, and to determine multidrug resistant isolates for possible intervention.

#### Materials and Methods:-

This study was carried out in Era's Lucknow Medical College & Hospital, Lucknow, which is a tertiary care hospital , in the month of August 2015. Ethical clearance was taken from the Institutional Ethics Committee.

A total of 60 hand swab samples were collected from 60 healthcare workers of the Hospital. Of all the healthcare workers, 50 (83.3%) were female nurses and 10 (16.7%) were male nurses, which were chosen randomly. Sterile cotton wool swabs, which were pre- moistened in sterile normal saline, were used to swab the interdigital spaces, dorsal and ventral aspect of hands of the participants. The media used in this study were Blood Agar, MacConkey

Agar and Mueller Hinton Agar. The swabs from hands were cultured on Blood agar and MacConkey agar plates. The plates were incubated at 35°C for 24-48 hrs. The bacterial isolates were identified using bacteriological procedures, involving microscopy, morphology, and biochemical tests.

All detected bacterial isolates were tested for antimicrobial susceptibility test by the standard Kirby-Bauer disc diffusion method, according to the Clinical Laboratory Standard Institute (CLSI) guidelines. The test organism was picked up with a sterile loop, suspended in peptone water and incubated at 37°C for 2 h. The turbidity of the suspension was adjusted to 0.5 McFarland's standard. It was then spread on the surface of Mueller-Hinton Agar (MHA) plate using sterile cotton swab. After drying the plates (37°C for 30 minutes) antibiotic discs were applied by sterile forceps. *Staphylococcus aureus* ATCC 25923 and *Escherichia coli* ATCC 25922, *P. aeruginosa* ATCC 27853 were used as controls during the study.

### Results:-

A total 60 hand swab sample, were collected from 60 healthcare workers of Era's Lucknow Medical College & Hospital, Lucknow, among which 50 (83.3%) were female nurses and 10(16.7%) were male nurses. Of the 60 samples analysed, 48 (80%) yielded bacterial growth, while 12 (20%) showed no bacterial growth. Out of the 48 isolates, 36 (75%) were Gram negative, and 12(25%) were Gram positive organisms. Also out of total 50(83.3 %)females nurses, 30(60 %) had Gram negative and 8 (16 %) had gram positive bacteria; while out of 10( 16.7 %)male nurses, 6( 60%) had Gram negative and 4 ( 40%) had Gram positive bacteria.(Table1)

TOTAL NO. (%)	TOTAL GRAM NEGATIVE	TOTAL GRAM POSITIVE
FEMALE NURSES- 50 (83.3)	30 (60%)	8 (16%)
MALE NURSES – 10 (16.7)	6 (60%)	4 (40%)

**TABLE 1:-** ( Distribution of Gram negative and Gram positive bacteria among female and male nurses)

Also out of total 38 bacterial isolates in female nurses,30(78.9 %) were Gram negative and 8 (21.05 %) were gram positive bacteria ;while out of 10 bacterial isolates in male nurses, 6 (60%) were Gram negative and 4 (40%) Gram positive bacteria.(Table 2)

SOURCE		TOTAL NUMBER OF ISOLATES	GRAM NEGATIVE	GRAM POSITIVE
HAND SWABS	FEMALE NURSES	38	30(78.9%)	8(21.05%)
	MALE NURSES	10	6(60%)	4(40%)

**TABLE 2:-** (Distribution of Bacteria among nurses, out of total bacterias isolated)

Of the 60 samples analyzed, bacterial isolates were seen in 48. Out of total 48, organisms were identified as 22( 45.8%) *Escherichia coli*, 4(8.3 %) *Pseudomonas aeruginosa* ,10(20.8 %) *Klebsiella pneumoniae* and 12 as *Staphylococcus spp*, of which 8(16.6 %) were *Staphylococcus aureus* and 4 ( 8.3 %) were *Coagulase Negative Staphylococcus(CONS)*.(Table 3)

TOTAL BACTERIAL ISOLATES	48
<i>Escherichia coli</i>	22(45.8%)
<i>Pseudomonas aeruginosa</i>	4(8.3%)
<i>Klebsiella pneumoniae</i>	10(20.8%)
<i>Staphylococcus aureus</i>	8(16.6%)
CONS	4(8.3%)

**Table 3-** Different bacterias isolated in the study

Out of 22( 45.8 %) *Escherichia coli* isolated, 20(90.9%)were in female nurses and 2(9.1%)were in male nurses. Out of 4( 8.3 %) *Pseudomonas aeruginosa*, all the 4(100%)were in female nurses and none was found in male nurse 0(0%).

Out of 10( 20.8 %) *Klebsiella pneumoniae*,6(60%)were in female nurses and 4(40%) were in male nurses.

12were *Staphylococcus spp*, of which 8(16.6 %) were *Staphylococcus aureus* and 4 ( 8.3 %) were CONS.

Out of 8(16.6%) *Staphylococcus aureus*, 6(75%) were in female nurse and 2(25%) in male nurses.

Out of 4 (8.3 %)CONS, 2(50%) were in female nurses and 2(50%) were in male nurses. (Table 4)

ORGANISMS	ORGANISMS FOUND IN FEMALE NURSES	ORGANISMS FOUND IN MALE NURSES
<i>ESCHERICHIA COLI</i> 22(45.8%)	20(90.9%)	2(9.1%)
<i>PSEUDOMONAS AERUGINOSA</i> 4(8.3%)	4(100%)	0(0%)
<i>KLEBSIELLA PNEUMONIAE</i> 10(20.8%)	6(60%)	4(40%)
<i>STAPHYLOCOCCUS AUREUS</i> 8(16.6%)	6(75%)	2(25%)
<i>CONS</i> 4(8.3%)	2(50%)	2(50%)

**Table 4-** Distribution of total Bacteria isolated among female and male nurses

Out of total 50(83.3%) female nurses, *Escherichia coli* were isolated from 20(40 %) female nurses, *Pseudomonas aeruginosa* were isolated from 4(8 %) female nurses, *Klebsiella pneumoniae* were isolated from 6( 12 %) female nurses; and *Staphylococcus spp* were isolated from 8(16 %) female nurses ; of which 6(75%) were *Staphylococcus aureus* and 2( 25 %) were *CONS*.

Out of total 10(16.7%) male nurses, *Escherichia coli* were isolated from 2(20 %) male nurses, *Pseudomonas aeruginosa* were isolated from 0( 0%) male nurse, *Klebsiella pneumoniae* were isolated from 4( 40 %) male nurses; again, *Staphylococcus spp* were isolated 4(40 %) male nurses ; of which 2(50%) was *Staphylococcus aureus* and 2(50 %) was *CONS*.(Table 5).

TOTAL FEMALE NURSES 50(83.3%)	<i>ESCHERICHIA COLI</i>	<i>PSEUDOMONAS AERUGINOSA</i>	<i>KLEBSIELLA PNEUMONIAE</i>	<i>STAPHYLOCOCCUS AUREUS</i>	<i>CONS</i>
	20(40%)	4(8%)	6(12%)	6(75%),OUT OF 8(16%)	2(25%),OUT OF 8(16%)
TOTAL MALE NURSES 10(16.7%)	2(20%)	0(0%)	4(40%)	2(50%),OUT OF 4(40%)	2(50%), OUT OF 4(40%)

**Table 5-** Distribution of bacterial isolates among total female and male nurses

The proportion of gram negative bacteria was high 36(75%),when compared to gram positive bacteria 12(25%),out of total 48 isolates. The antibiotic sensitivity pattern were as follows:-

*Escherichia coli* showed 100% sensitivity to Ciprofloxacin, Doxycycline, Amikacin, Gentamycin, Levofloxacin ;but were resistant to Amoxicillin/ clavulanic acid(81.8%), Ceftriaxone(72.7%)and Co-Trimoxazole(63.6%).Total ESBL producers were 72.72%.

*Pseudomonas aeruginosa* showed 100% sensitivity to Ciprofloxacin, Levofloxacin, Piperacillin/Tazobactam, Imipenem; but were resistant to Ceftazidime(75%), Cefepime(50%), Gentamycin(25%), Cefotaxime(75%).

*Klebsiella Pneumoniae* showed 100% sensitivity to Ciprofloxacin, Doxycycline, Amikacin, Gentamycin, Levofloxacin ; but were resistant to Amoxicillin/ clavulanic acid(60%), Ceftriaxone(60%) and Co-Trimoxazole(50%).Total ESBL producers were 60%.

*Staphylococcus aureus* showed 100% sensitivity toAmikacin; but were resistant to Cefoxitin, Levofloxacin(12.5%),Amoxicillin/ clavulanic acid (25%),Ceftriaxone(62.5%),Clindamycin(25%),Erythromycin(50%),Ciproflaxacin(50%). Total MRSA was 12.5%. *CONS* showed 100% sensitivity to Cefoxitin, Levofloxacin, Amikacin; but were resistant to Amoxicillin/ clavulanic acid (25%),Ceftriaxone(75%),Clindamycin(25%),Erythromycin(50%),Ciproflaxacin(50%). All of the *CONS* were Methicillin susceptible.(Table 6,7,8).

ORGANISMS	AMC	CIP	DO	AK	G	LE	CTR	COX	CAZ	CPM	PIT	IPM	CTX
<i>ESCHERICHIA COLI</i> -22(45.8%)	4(18.18%)	22(100%)	22(100%)	22(100%)	22(100%)	22(100%)	6(27%)	8(36%)					
<i>PSEUDOMONAS AERUGINOSA</i> -4(8.3%)		4(100%)			3(75%)	4(100%)			1(25%)	2(50%)	4(100%)	4(100%)	1(25%)
<i>KLEBSIELLA PNEUMONIAE</i> -10(20.8%)	4(40%)	10(100%)	10(100%)	10(100%)	10(100%)	10(100%)	4(40%)	5(50%)					

**Table:6-** Antibiotic Sensitivity Pattern Of Gram Negative Bacteria

ORGANISMS	AMC	CIP	AK	LE	CTR	CX	CD	E
<i>STAPHYLOCOCCUS AUREUS</i> - 8(16.6%)	6(75%)	4(50%)	8(100%)	7(87.5%)	3(37.5%)	7(87.5%)	6(75%)	4(50%)
<i>CONS</i> - 4(8.3%)	3(75%)	2(50%)	4(100%)	4(100%)	1(25%)	4(100%)	3(75%)	2(50%)

**Table : 7-** Antibiotic Sensitivity Pattern Of Gram Positive Bacteria

ORGANISMS	AMC	CIP	DO	AK	G	LE	CTR	COT	CAZ	CPM	PIT	IPM	CTX	CX	CD	E
<i>ESCHERICHIA COLI</i> -22(45.8%)	4(18.18%)	22(100%)	22(100%)	22(100%)	22(100%)	22(100%)	6(27%)	8(36%)								
<i>PSEUDOMONAS AERUGINOSA</i> -4(8.3%)		4(100%)			3(75%)	4(100%)			1(25%)	2(50%)	4(100%)	4(100%)	1(25%)			
<i>KLEBSIELLA PNEUMONIAE</i> -10(20.8%)	4(40%)	10(100%)	10(100%)	10(100%)	10(100%)	10(100%)	4(40%)	5(50%)								
<i>STAPHYLOCOCCUS AUREUS</i> - 8(16.6%)	6(75%)	4(50%)		8(100%)		7(87.5%)	3(37.5%)							7(87.5%)	6(75%)	4(50%)
<i>CONS</i> - 4(8.3%)	3(75%)	2(50%)		4(100%)		4(100%)	1(25%)							4(100%)	3(75%)	2(50%)

**Table :8-** Antibiotic Sensitivity Pattern Of Both Gram Positive And Gram Negative Bacteria

(AMC=30mcg, CIP=5mcg, DO=30mcg, AK=30mcg, G=10mcg, LE=5mcg, CTR=30mcg, COT=25mcg, CAZ=30mcg, CPM=30mcg, PIT=100/10mcg, IPM=10mcg, CTX=30mcg, CX=30mcg, CD=2mcg, E=15mcg).

### Discussion:-

A total of 60 healthcare workers, which included 50 female nurses and 10 male nurses, were included in this study, which were chosen randomly. Out of the 60 hand swabs samples analysed, 48 (80%) yielded bacterial growth, while 12 (20%) showed no bacterial growth.

Out of the 48 isolates, 36 (75%) were all Gram negative, and 12 (25%) were gram positive organisms. Of 48 isolates, organisms were identified as 22 (45.8%) *Escherichia coli*, 4 (8.3%) *Pseudomonas aeruginosa*, 10 (20.8%) *Klebsiella pneumoniae* and 12 as *Staphylococcus spp.*, of which 8 (16.6%) were *Staphylococcus aureus* and 4 (8.3%) were *Coagulase Negative Staphylococcus (CONS)*. Among the gram negative organisms, 16 (72.72%) ESBL producers were *Escherichia coli* and 6 (60%) ESBL producers were *Klebsiella pneumoniae*. Out of 8 *S. aureus*, 1 (12.5%) was MRSA. Among *CONS*, all of them were Methicillin sensitive.

This can be compared to a study carried out by Maheshwari *et al.* [7], which included 70 HCWs, comprising 20 doctors, 20 nurses, 20 operation theatre (OT) technicians and 10 were laboratory technicians, from whom a total of 140 swabs were collected; and the organisms which were isolated were *S. aureus* in 13 (18.6%) and *coagulase negative staphylococcus* in 44 (63%).

Out of 13, *S. aureus* isolates 6 (46.2%) were MRSA. Gram negative bacteria (GNB) were isolated in 22 (31.5%), out of which 15 (68.2%) were ESBL producers. Among the 15 ESBL producers, 9 were *Klebsiella pneumoniae*, 5 were *Escherichia coli* and 1 was *Proteus mirabilis*.

Sarfraz *et al.* [8] conducted a similar type of study, comparing doctors and nurses of clinical department; and doctors and staff of non-clinical department. Organisms isolated were *Staphylococcus aureus*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, *Coagulase negative Staphylococcus spp.*, *Acinetobacter lwoffii*, *Escherichia coli* and *Corynebacterium spp.*, from hands of clinical staff (doctors + nurses). Organisms isolated from non-clinical staff was *Acinetobacter lwoffii*, *Coagulase negative Staphylococcus spp.*, *Staphylococcus aureus*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa* and *Micrococcus spp.*

Survana Sande *et al.* [9], also showed in their study, a high rate of bacterial colonization in hand swab samples of HCWs, and proved the importance of hand washing, by showing very low rate of bacterial colonization in hand swab samples after hand washing. In their study, out of total 150 samples collected from Nursing staffs before hand washing, growth of microorganisms was observed in 107 (71.3%) samples and no growth in 43 (28.7%) samples. No growth was obtained in 128 (85.3%) samples and single type of growth in 22 (14.7%) samples, after hand washing.

Of the 60 samples analyzed in our study, maximum samples showed growth of *Escherichia coli*. *E. coli* is Gram-negative, aerobic and facultative anaerobic bacteria [10]. *E. coli* is an emerging nosocomial pathogen causing serious problems in health care settings [11]. This species leads to both intestinal and extraintestinal infections in humans and many animals. Currently, six major groups of intestinal pathogenic *E. coli* (IPEC) have been recognized: enteropathogenic *E. coli* (EPEC), enteroaggregative *E. coli* (EAEC), Shiga toxin-producing *E. coli* (STEC), enterotoxigenic *E. coli* (ETEC), enteroinvasive *E. coli* (EIEC), and diffusely adherent *E. coli* (DAEC). Three types of extraintestinal pathogenic *E. coli* (ExPEC) are also recognized, including neonatal meningitis-causing *E. coli* (NMEC), sepsis-causing *E. coli* (SEPEC), and uropathogenic *E. coli* (UPEC), associated with meningitis in newborns, systemic infections, and urinary tract infections (UTIs), respectively [12].

In our study, *Escherichia coli* showed 100% sensitivity to Ciprofloxacin, Doxycycline, Amikacin, Gentamycin, Levofloxacin; but were resistant to Amoxicillin/clavulanic acid (81.8%), Ceftriaxone (72.7%) and Co-Trimoxazole (63.6%). Total ESBL producers were 72.72%.

*Pseudomonas aeruginosa* showed 100% sensitivity to Ciprofloxacin, Levofloxacin, Piperacillin/Tazobactam, Imipenem; but were resistant to Ceftazidime (75%), Cefepime (50%), Gentamycin (25%), Cefotaxime (75%).

*Klebsiella Pneumoniae* showed 100% sensitivity to Ciprofloxacin, Doxycycline, Amikacin, Gentamycin, Levofloxacin; but were resistant to Amoxicillin/clavulanic acid (60%), Ceftriaxone (60%) and Co-Trimoxazole (50%). Total ESBL producers were 60%.

*Staphylococcus aureus* showed 100% sensitivity to Amikacin, (87.5%) to Cefoxitin and Levofloxacin, (75%) to Amoxicillin/ clavulanic acid, (75%) to Clindamycin, (50%) to Erythromycin, (50%) Ciprofloxacin, and least sensitive to Ceftriaxone (37.5%). 1 (12.5%) was MRSA.

*CONS* were (100%) sensitive to Cefoxitin, Amikacin, Levofloxacin; (75%) to Amoxicillin/ clavulanic acid, Clindamycin; (50%) to Erythromycin, Ciprofloxacin, and least sensitive to Ceftriaxone (25%). All of the *CONS* were Methicillin sensitive.

In a study carried by Edem EN *et al.* [13], the antibiotic sensitivity pattern of the *Staphylococcus aureus* showed 60% to Oxacillin, 80% to Clindamycin, 43.3% to Erythromycin, 40% to Ceftriaxone, 77% to Ciprofloxacin, 23.3% to Trimethoprim Sulphamethoxazole and 73.3% to Amoxicillin clavulanic acid. 13% of the *Staphylococcus aureus* were found to be inducible clindamycin resistant. The antibiotic sensitivity pattern of *Staphylococcus epidermidis* showed 56.2%, 75%, 19%, 81.3%, 12.5% and 68.8% to Erythromycin, Clindamycin, Ceftriaxone, Ciprofloxacin, Trimethoprim Sulphamethoxazole and Amoxicillin clavulanic acid, respectively. 6.25% of the *Staphylococcus epidermidis* were found to be inducible Clindamycin resistant. *Escherichia coli* showed 100% sensitivity to Ciprofloxacin, Ceftriaxone, Gentamycin, Ceftazidime and Cefotaxime, but were resistant to Amoxicillin clavulanic acid and Cefpodoxime. It was non-ESBL producing. *Proteus mirabilis* showed 100% sensitivity to Gentamycin, Ceftriaxone, Amoxicillin clavulanic acid and Cefotaxime. It was ESBL producing.

MRSA isolates showed 0%, 50%, 91%, 33.3% and 75% sensitivity to Oxacillin, Erythromycin, Clindamycin, Ceftriaxone, Ciprofloxacin, Trimethoprim Sulphamethoxazole and Amoxicillin clavulanic acid, respectively, and 16.6% of the MRSA isolates were inducible Clindamycin resistant.

Chaka *et al.* [14], in their study took samples from dominant hands of staff nurses, pediatric residents and medical interns and their cell phones. *Staphylococcus aureus* strains isolated from hand swabs were resistant to oxacillin, vancomycin and ceftazidime in 46%, 24% and 44% respectively. The resistance pattern of *Staphylococcus aureus* from cell phone isolates were 51.6%, 14% and 51% respectively for oxacillin, vancomycin and ceftazidime. *CONS* isolated were also resistant to commonly prescribed antibiotics.

Kumar *et al.* [15] in their study showed that almost 25% of healthcare workers are stable nasal carriers and 30%-50% of them possess the bacteria in their hands. Tammelin *et al.* [16] also showed in their study that 50.7% of healthcare workers carry bacteria in their nose and 26.3% in their hands.

Several studies have shown that most of the bacteria that cause nosocomial infections are those that have developed resistance to antibiotics used in treating those [17].

### Conclusion:-

Since the group of individuals under this study were healthcare workers, their interaction and exposure to hospital environment could cause major risks in transmitting to hospital patients and spreading nosocomial infections.

So, by simple measure of hand washing, various infections can be controlled.

Healthcare workers should wash their hands regularly with antiseptic soap, or disinfect the hand by rubbing with alcohol solution.

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