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Bacterial Prevalence and Resistance to Antimicrobial Agents in Southwest, Saudi Arabia

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ABSTRACT

One hundred and eighty eight organisms were isolated from clinical specimens (71 isolates from urine, throat swabs (40), stool (39) pus (17), blood (14), wound swabs (7) collected from laboratories of hospitals and polyclinics distributed in Najran Area, Saudi Arabia, between February 2010 to November 2011. Bacteria were identified by Gram staining and biochemical tests, and antibiotic sensitivities tested by the disc diffusion method at microbiology laboratory, Najran University. The most prevalent bacteria isolated were E. coli (35.63%) followed by Klebsiella pneumoniae (18.08%), Staph. aureus (14.89%), Salmonella spp. (13.29%), Pseudomonas aeruginosa (6.91%), Streptococcus pneumoniae (5.31%), Shigella spp (3.19%), Enterococcus faecalis (1.59%) and Proteus mirabilis (1.06%). The multi-drug resistance rates (MDR) among common isolates were Pseudomonas aeruginosa (38.46%) followed by Klebsiella pneumoniae (32.35%), Staph. Aureus (32.14%) and E. coli (31.34%). The overall multi-drug resistance rate among isolates was high (28.72%).

Keywords: bacteria, prevalence, antimicrobial, resistance.

INTRODUCTION

Antibiotic resistance has become a major clinical and public health problem. We are currently faced with (multi) resistant bacteria that are difficult and sometimes impossible to treat, Levy, S.B. (2002). The tremendous therapeutic advantage afforded by antibiotics is being threatened by the emergence of increasingly resistant strains of microbes, Livermore, D.M. (2005). The problem has recently been worsened by the steady increase in multi-resistant strains and by the restriction of antibiotic discovery and development programs, Levy S.B (2002). The widespread use of antibiotics both inside and outside of medicine is playing a significant role in the emergence of resistant bacteria, Bacon, D.J. *et al*. (2000). Antimicrobials have transformed our ability to treat many infectious diseases that were killers only a few decades ago. The increasing use of antimicrobials in humans, animals, and agriculture has resulted in many pathogens developing resistance to these powerful drugs, Sakharkar, MK. *et al*. (2009). Many diseases are increasingly difficult to treat because of the emergence of drug-resistant organisms, including bacteria such as staphylococci, enterococci, and *Escherichia coli;* respiratory infections such as tuberculosis and influenza; food-borne pathogens such as Salmonella and *Campylobacter;* sexually transmitted organisms such as *Neisseria gonorrhoeae*, Boyd, D. *et al*. (2004) & Chambers, H. F. (2005). The problem of antimicrobial (drug) resistance requires a multi-pronged research strategy on many aspects of antimicrobial (drug) resistance, from basic research on how microbes develop resistance to clinical trials that translate research from lab findings to potential treatments, Esposito,

S. and Leone, S. (2007). Bacteria have developed resistance to all different classes of antibiotics discovered to date. The most frequent type of resistance is acquired and transmitted horizontally via the conjugation of a plasmid, Streit, JM. *et al*. (2004). In recent times new mechanisms of resistance have resulted in the simultaneous development of resistance to several antibiotic classes creating very dangerous multidrug-resistant (MDR) bacterial strains, some also known as "superbugs" Nienke van de Sande-Bruinsma, *et al*. (2008). The need for new antimicrobial agents is greater than ever because of the emergence of multidrug resistance in common pathogens, the rapid emergence of new infections, and the potential for use of multidrug-resistant agents in bioweapons, Kent Peters, N. *et al*. (2008). Controlling the spread of resistance requires the collaboration of several participants such as Veterinary, Medical, and Public Health Communities, Angulo, F.J. *et al*. (2004). Multidrug resistant organisms (MDROs) are resistant to one or more classes of antimicrobial agents and the knowledge of susceptibility pattern is helpful in selecting the empirical therapy and improving the likelihood of a satisfactory outcome for patient, Sameera M. *et al*. (2010). The objective of this study was to determine bacterial pathogens prevalence and to assess the multi-drug resistant (MDR) strains to different antibiotics in southwest, Saudi Arabia.

MATERIALS AND METHODS

Sample Collection

Mid-stream urine, stool, pus, wound swabs, throat swabs and blood specimens were collected aseptically for bacteriological examination from laboratories of hospitals and polyclinics distributed in Najran Area between February 2010 to November 2011. Handling, transporting and storing of collected samples were made at refrigeration temperature.

Isolation and Identification

Urine, pus, wound swabs, throat swabs and blood specimens were cultured onto blood agar and MacConkey agar media. Stool specimens were inoculated onto Salmonell-Shigella agar (including a subculture of Selenite-F broth), Xylose Lysine deoxycholate and Mac Conkey agar media then incubated at 37ºC for 18-24 hours. Bacteriological smears were prepared from the growing colonies then stained with gram stain for morphological identification. All the bacterial isolates were preserved on nutrient agar slants at 4ºC and subcultured periodically. The obtained pure cultures were identified biochemically, Holt, J. G. *et al*. (1994) and Pelczar, M. J. *et al*. (1999).

Antimicrobial Susceptibility Test

Antimicrobial susceptibility pattern was performed using disc diffusion method on Muller Hinton agar plate (15, 16). The isolates were tested against ampicillin (10 ug), ceftazidime (30ug), gentamicin (10 ug), imipenem (10 ug), ciprofloxacin (5 ug), ceftriaxone (30 ug), amikacin (30 ug), tetracycline (30 ug) and trimethoprim-sulfamethoxazole. (25 ug). The proportion of susceptible organisms was calculated as the sum of susceptible isolates relative to the total number of organisms tested. The organism considered as multidrug resistant if it is resistant to three or more antimicrobials.

RESULTS AND DISCUSSION

Bacteriological examination revealed that 188 organisms were isolated from clinical specimens. 71 isolates from urine, throat swabs (40), stool (39) pus (17), blood (14), wound swabs (7) (Tables 1-6). As shown in Table 1, of 71 isolates recovered from urine specimens, 45 were E. coli (63.38%) of which 14 (31.11%) were multi- drug resistant, followed by Klebsiella pneumoniae (23.94%) with

MDR rate (29.41%). Similar findings were cited in previous studies (17, 18). Examination of throat swabs revealed that the most prevalent organism wasK lebsiella pneumoniae (27.50%) and antimicrobial resistance (36.36%). Another study, Hörü Gazi, *et al*. (2004), reported that the most prevalent organisms isolated from throat swabs in Manisa, Turkey were Streptococcus pneumoniae (15.8%).

Table 1: Bacterial species isolated from urine specimens:

Sample	Bacterial isolates	NO	%	Sensitive		MDR	
Urine				No	%	No	%
	E. coli	45	63.38	31	68.88	14	31.11
	Klebsiellapneumoniae	17	23.94	12	70.58	5	29.41
	Enterococcus faecalis	3	4.22	2	66.66	1	33.33
	Pseudomonas aeruginosa	3	4.22	1	33.33	2	66.66
	Staph. aureus	3	4.22	2	66.66	1	33.33
	Total	71		48	67.60	23	32.39

Table 2: Bacterial species isolated from throat swabs:

Sample	Bacterial isolates	NO	%	Sensitive		MDR	
				No	%	No	%
Throat swab	Klebsiellapneumoniae	11	27.50	7	63.63	4	36.36
	E. coli	10	25.00	8	80.00	2	20.00
	Pseudomonas aeruginosa	7	17.50	5	71.42	2	28.57
	Streptococcus pneumonia	6	15.00	4	66.66	2	33.33
	Staph. aureus	6	15.00	5	83.33	1	16.66
	Total	40		29	72.50	11	27.50

Table 3: Bacterial species isolated from stool specimens:

Sample	Bacterial isolates	NO	%	Sensitive		MDR	
				No	%	No	%
Stool	Salmonella sp.	24	61.53	21	87.50	3	12.50
	Staph. aureus	7	17.94	6	85.71	1	14.28
	Shigella spp.	6	15.38	5	83.33	1	16.66
	Proteus mirabilis	2	5.12	2	100.00	0	00.00
	Total	39		34	87.17	5	12.82

Table 4: Bacterial species isolated from pus specimens*:*

Sample	Bacterial isolates	NO	%	Sensitive		MDR	
				No	%	No	%
Pus	E. coli	8	47.05	4	50.00	4	50.00
	Staph. aureus	5	29.41	2	40.00	3	60.00
	Klebsiellapneumoniae	2	11.76	2	100.00	0	0.00
	Pseudomonas aeruginosa	2	11.75	1	50.00	1	50.00
	Total	17		9	52.94	8	47.05

Table 5: Bacterial species isolated from blood specimens:

Sample	Bacterial isolates	NO	%	sensitive		MDR	
				No	%	No	%
Blood	Staph. aureus	5	35.71	3	60.00	2	40.00
	Klebsiella pneumonia	4	28.57	3	75.00	1	25.00
	Salmonella spp.	1	7.14	1	100.00	0	0.00
	Streptococcus pneumonia	4	28.57	3	75.00	1	25.00
	Total	14		10	71.42	4	28.57

Table 6: Bacterial species isolated from wound swabs.

Sample	Bacterial isolates	NO	%	sensitive		MDR	
				No	%	No	%
Wound swab	E. coli	4	57.14	3	75.00	1	25.00
	Staph. aureus	2	28.57	1	50.00	1	50.00
	Pseudomonas aeruginosa	1	14.28	0	00.00	1	100.00
	Total	7		4	57.14	3	42.85

Of 39 organisms isolated from stool samples, 24(61.53%) were Salmonella spp. with high susceptibility to antimicrobials (87.50%) (Table3). These results are consistent with a previous study, George Samonis *et al*. (2010). E. coli was the most common organisms isolated from pus (47.05%) and resistant rate (50.00%) followed by Staph. aureus (29.41%) with resistance (60.0%). Similar results were cited, Mohanty, S. *et al*. (2004) (21). Of 14 organisms obtained from blood specimens, 5 isolates was Staph., aureus (35.71%) and resistance rate was (40%) (Table 5). Similar observations were previously recorded, Stephen G. Weber, *et al*. (2009). Of 7 isolates obtained from wound swabs, 4(57.14%) were E.coli and resistance rate was (25%). 2(28.57%) isolates were Staph.aureus and resistance rate was (50%) (Table6). These results approximately agree with those recorded by, Alireza Ekrami and Enayat Kalantar (2007). Our study revealed that the most prevalent bacteria isolated were E. coli (67 isolates, 35.63%) followed by Klebsiellapneumoniae (34 isolates, 18.08%), Staph. Aureus (28 isolates, 14.89%) Salmonella spp. (25 isolates, 13.29%), Pseudomonas aeruginosa (13 isolates, 6.91%), Streptococcus pneumoniae (10 isolates, 5.31%), Shigella spp. (6 isolates, 3.19%), Enterococcus faecalis (3 isolates, 1.59%), Proteus mirabilis (2 isolates, 1.06%) (Table7).

Table 7: Overall Bacterial prevalence and susceptibility pattern.

Bacterial isolates	NO	%	Sensitive		MDR	
			NO	%	NO	%
E. coli	67	35.63	46	68.65	21	31.34
Klebsiellapneumoniae	34	18.08	23	67.64	11	32.35
Staph. aureus	28	14.89	19	67.85	9	32.14
Salmonella spp.	25	13.29	22	88.00	3	12.00
Pseudomonas aeruginosa	13	6.91	8	61.53	5	38.46
Streptococcus pneumoniae	10	5.31	7	70.00	3	30.00
Shigella spp.	6	3.19	5	83.33	1	16.66
Enterococcus faecalis	3	1.59	2	66.66	1	33.33
Proteus mirabilis	2	1.06	2	100.00	0	00.00
TOTAL	188		134	71.27	54	28.72

Similar results were previously recorded, George Samonis *et al*. (2010); Potaschmacher, L.O. *et al*. (1979) and Rotimi, VO. *et al*. (1998). Pseudomonas aeruginosa, Escherichia coli, Klebsiella pnemoniae and Enterobacter were the most frequently isolated organisms in an adult ICU at a tertiary care hospital in Riyadh, Saudi Arabia, Sameera, M. *et al*., 2010 and Jones *et al*., 2004, assimilated in vitro susceptibility data from over 220000 isolates from ICUs in five countries (France, Germany, Italy, Canada, and the United States) over the period 2000 to 2002. Eltahawy, AT. and Khalaf, RM., evaluated 100 isolates and found that P aeruginosa, K pneumonia and E. coli were the most commonly isolated from teaching hospital in Saudi Arabia. Regarding the in vitro sensitivity

of isolates to different antimicrobial agents, the organism is considered as multidrug resistant if it is resistant to three or more antimicrobials. Susceptibility test showed that the multi-drug resistance rate among the most prevalent isolates were Pseudomonas aeruginosa (38.46%), Klebsiella pneumoniae (32.35%), Staph. aureus (32.14%) and E. coli (31.34%) (Table 7). These results approximately agree with those recorded by, Narten, Maike *et al*., 2012 and Sameera M. *et al*., 2010, recorded significant resistance of cefotaxime to E coli (24%-54%). Mohanty *et al*., 2004, found that *resistance* in S. aureus was 38.56%, high level aminoglycoside *resistance* was observed in 53.3% of enterococci and 66.75% of the gram negative bacilli in North India. The overall multi-drug resistance rate was 28.72%. Higher number of resistant bacteria seen in Saudi Arabia might be due to greater antibiotic consumption, Alireza Ekrami and Enayat Kalantar (2007). Asghar and Faidih, 2010, performed a study in Makkah. They reported much higher resistance rate among gram negative bacteria in comparison with other countries in the world which necessitates implementation of monitoring program. Therefore developing nationwide antibiotic policy and guidelines is essential to limit multidrug resistance and to maintain low level of resistance to newer antibiotics in Saudi Arabia.

European

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Transparency declarations

The authors have none to declare.

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ARABIC SUMMARY

مدى انتشار ومقاومة البكتريا للمضادات الحيوية بجنوب غرب المملكة العربية السعودية

السيد السعيد مسعود و محمد عصمت مهدي و أحمد محمد عصمت
قسم العلوم الطبية التطبيقية- كلية المجتمع-جامعة نجران –المملكة العربية السعودية

تم عزل 188 عترة بكتيرية من عينات سريرية (71 معزولة من البول،40 معزولة من مسحات الحلق،39 معزولة من البراز،17 معزولة من عينات صديدية،14 معزولة من عينات دم ،7 معزولة من مسحات جروح) جمعت من مختبرات المستشفيات والمستوصفات الطبية المنتشرة بمنطقة نجران خلال الفترة من فبراير 2010 حتى نوفمبر 2011. وقد صنفت المعزولات كيموحيويا كما تم عمل اختبار حساسية لهذه المعزولات. وقد أظهرت النتائج أن البكتريا الأكثر انتشارا هي الايشيرشيا كولاي (35.36%) تلتها الكلبسيلا نيموني (18.08%) ،العنقودية الذهبية (14.89%) ،السالمونيلا (13.29%) ،السيدوموناس إيروجينوزا(6.91%)،الاستربتوكوكس نيموني (5.31%) ،الشيجلا (3.19%) ، الانتيروكوكس فيكاليس (1.59%) ثم البروتيوس ميرابيليس (1.06%). أظهر اختبار الحساسية أن السيدوموناس ايروجينوزا هي أكثر أنواع البكتريا مقاومة للعديد من المضادات الحيوية(MDR) بنسبة 38.46% تلتها الكلبسيلا نيموني (32.35%) ، العنقودية الذهبية (32.14%) ثم الايشيرشيا كولاي (31.34%). أوضحت النتائج أن المعدل الإجمالي لمقاومة البكتريا المتعددة (MDR) للمضادات الحيوية بجنوب غرب المملكة كانت عالية حيث بلغت النسبة 28.72%.