Science Journal of Microbiology
ISSN: 2276-626X
http://www.sjpub.org/sjmb.html

Published By Science Journal Publication

Research Article Volume 2012, Article ID sjmb-112, 13 Pages, 2012. doi: 10.7237/sjmb/112

A Study of Epidemiology and Etiology of Bacteremia Isolates from Patients in Riyadh City of Saudi Arabia

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Accepted 27th November, 2012

ABSTRACT

Aim: Detection of the interface between etiological agents of bacteremia and epidemiology.
Methods: A total of 164 blood samples were collected from patients infected by bacteremia in King Khalid University Hospital, Riyadh, Saudi Arabia. Special bottles of BACTEC/ALERT PF microbial detection System (BIOMÉRIEUX brand) were used as blood culture bottles with antimicrobial removal systems. Bacterial species were identified using cultural, morphological and standard biochemical tests e.g. the oxidase, catalase and Indole tests.
Results: Elderly category of age was the highest (38.3%), compared to the adult's category (33.2%), then came the other categories (infants, pediatrics, teenagers) which were statistically equal (13.2, 11.0, and 4.3% respectively). The average of infected people in an Inpatient Departments (38.7%) was higher incorporeal than infected people in an Outpatient Departments (11.3%). Identification of bacterial genus and species according to the biochemical definition prospects was carried out in Hospital's Bacteriology Lab. Pathogens were identified in 173 organisms of Gram Positive, Gram Negative bacteria and yeasts. The patients can be infected by one or more than one pathogenic bacteria. The statistical analysis showed a non significant difference between the infected patients by either G^-ve or G^+ve in both males and females.
Conclusion: Patients can be infected by one bacterium, more than one bacterium or yeast. *E. coli* and *Staphylococcus* sp. exhibited significant differences in comparison with other genera (G^-ve and G^+ve respectively) isolated from blood.

KEYWORDS: Epidemiology, Etiology, age categories, Bacteremia, E. coli, Staphylococcus

INTRODUCTION

Blood is still the richest environment for many bacteria to grow to cause bacteremia. It leads to the possibility of microbial infection, serious blood diseases which are incurable and sometimes transmitted in the present age through blood transfusions (Volk et al., 1986) and they commonly spread in hospital (Mylonakis et al., 2006).

For reviewing the present work, a variety of studies was addressing the issue. Comparison between episodes of bacteremia and fungemia in children and adults with cancer to assess differences in etiology (Rahbar et al., 2005), risk factors (Llop et al., 2001), outcome (Krupova et al., 1998) and determination whether the organisms in the bloodstream originated from the patient's own flora (von Eiff et al., 2001) were reported.

Etiological agents of Bacteremia were studied by many researchers, who confirmed that *Neisseria meningitidis* was the most common species in community-acquired infections, and staphylococci predominated in hospital-acquired episodes (Gray et al., 2001), whereas Rahbar et al., (2005), reported that $Gram^+$ve cocci, including coagulase-negative staphylococci, *Staph. aureus, Streptococcus pneumoniae* and $Gram^-$ve bacilli, particularly *P. aeruginosa*, were responsible of Bacteremia isolates. While, results from Berezin and Iazzetti (2006) showed that the most common etiologic agent was *S. pneumoniae*.

From previous studies the relationship between Bacteremia and serious diseases with the causal agent was reported in Seydi et al., (2005) in cases of *E. coli* bacteremia was associated with meningitis and AIDS, Ekkelenkamp et al., (2007) reported that *Staph. aureus* bacteremia causes *Staph. aureus* bacteriuria, otherwise, Alamgir et al., (2006) found

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that gastrointestinal and genitourinary are sources for *E. coli* bacteremia.

The aim of this study is to detect the interface between etiological agents of bacteremia and epidemiology. To reach the goal, we designed our research through the study of the relationship between bacteremia and neither category of ages in both genders, Inpatient - Outpatients departments nor isolated gram negative and positive bacteria with which of those isolates are dominant in bloodstream.

MATERIALS AND METHODS

Samples Collections

In King Khalid University Hospital in Riyadh from March to July 2007, a total of 164 blood samples were collected from community and hospitalized patients, as well as collected clinical data from hospitalization files.

Blood culture bottles with antimicrobial removal systems were recommended for patients who develop fever while on antibiotics. Special bottles of BACTEC/ALERT PF microbial detection System (BIOMÉRIEUX brand) were used, each bottle contains suitable nutritional and environmental conditions for organisms commonly encountered in blood infections, and each bottle was spiked with 10 ml of patients' blood, to determine if microorganisms are present in blood taken from a patient suspected of having bacteremia/fungemia. Bottles were mixed and loaded onto their respective instruments as per the manufacturer's instructions. Antimicrobial removal was evaluated on the basis of time for detection of organism growth, for up to 7 days of incubation.

Specimens were cultured on blood agar; chocolate agar and MacCkonky in CO_2, O_2 incubators, and the plates were incubated overnight at 37℃.

Identification of bacterial isolates: Identification was carried out in the clinical bacteriology laboratory of the hospital. Bacterial species were identified using cultural, morphological and standard biochemical tests e.g. the oxidase, catalase and Indole tests were performed (Cheesbrough, 2000, Holt, et al., 2000, Shehabi, et al., 2004 and Abo El-Dahab, et al., 2011). To support the identification process**,** an automated system microscan was used, special panels for Gram^{+}ve and Gram^{-}ve (have 21 wells and 34 wells respectively), some wells dedicated to special biochemical reaction and some wells dedicated to some important antibiotics.

Statistical analysis

Data was analyzed by (SPSS, 2006) Program: Frequencies and percentages, averages and standard variations, variations analysis and Dunkin test.

RESULTS

Identification of Bacteremia Isolates

According to the standard tests recommended for the identification, the following Gram^{-}ve bacteria genera and species had been identified: *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Protus mirabilis*, *Acinetobacter lwoffi*, *A. baumannii/haemolyticus*, *Enterobacter agglomerans*, *En. cloacae*, *Citrobacter koseri*, *Citro. freundii*, *Alcaligenes* sp., *Alca. xylosoxidans* subsp. *xylosoxidans*, *Haemophilus influenzae, Moraxella catarrhalis*, *Achromobacter xylosoxidans* subsp. *xylosoxidans, Brucella* sp., *Salmonella* sp., *S.* sero group D1, *Ochrobactrum anthropi, Serratia marcescens*.

The following Gram Positive bacteria genera and species had been identified: *Streptococcus pneumonia, S. salivarius, S. viridans, S. mitis, S. sanguis, Streptococcus* Group A, B and D, *Staphylococcus aureus, Staph. simulans, Staph. hominis* subsp*. hominis, Staph. haemolyticus, Staph. epidermidis, Staph. auricularis, Staph. sciuri, Staph. xylosus, Staph. capitis* subspecies *ureolyticus, Staph. hominis* subsp*. novobiosepticus,* MRSA, *Enterococcus faecalis, Bacillus* spp., *Diphtheroids* sp., *Micrococcus* sp. Also some isolates of yeast has been detected.

Relationship between bacteremia and age categories

Statistical analysis illustrated that no significant differences ($p<0.0001$) between infected males and female with bacterimia, but there was a significant effect in the age categories, in which the percentage of elderly was the highest (38.3%), compared to the adults category (33.2%), then came the other categories (infants, pediatrics, teenagers) which were statistically equal (13.3, 11.0, 4.2% respectively), this means that there was no significant difference between them (Table 1, Figure 1).

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Age Categories / Sex	Infant		Pediatric		Teenagers		Adults		Elderly		
	0-2		3-11		12-21		22-60		> or = 61		
	No.	%	No.	%	No.	%	No.	%	No.	%	
Male	16	9.7	13	8	7	4.2	20	12.2	30	18.3	10.42 ± 4.90
Female	6	3.6	5	3	0	0	34	21	33	20	9.59 ± 9.36
Mean%± SD	6.4^{b}±3.23		5.78^{b} ± 2.45		2.25^{b} ± 2.59		16.45^{a} ± 4.90		19.15^{a} ± 0.98		

Table (1): The distribution of gender, males and females according to age categories and averages of infection (± standard variations) and variations analysis.

ANOVA

SOV	df	Mean Square	F- value	Sig.
Age	4	216.198	21.42	****
Sex	1	3.444	0.341	NS
Error	14	10.09		

*** P < 0.0001 NS = Non Significant

Means with no common superscript are significantly different (p < 0.05) (SPSS, 2006)

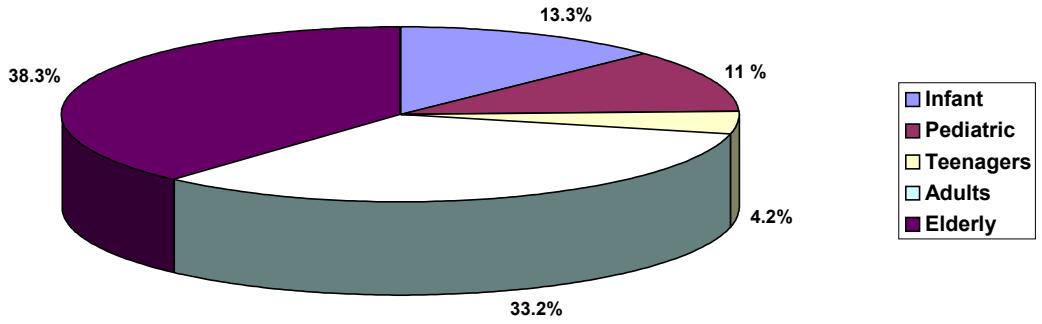

Fig. (1): The percentage of patients infected by bacteremia according to age categories.

Relationship between Bacteremia and Departments

The percentage of infected males in an Inpatient Department was more than the percentage of infected females, as much as 39.6%, and 37.8% respectively.

In an Outpatients Department the percentage of infected male was 12.2%, while % of infected female was 10.4%, so the total percentage of infection in an Inpatient and Outpatient departments was 77.4%, 22.6% respectively.

Table (2) and Figure (2) below illustrated the following: the significant diversity (P < 0.0001) in the percentage of infection by bacteremia between Inpatient and Outpatient Departments, in which the average of infected people in an Inpatient Department was higher incorporeal than infected people in an Outpatient Departments 38.7% and 11.3%respectively.

Table (2): Relationship between numbers of infected males and females and Departments

Sex / Department	Male		Female		Mean% ± SD
	No	%	No	%	
Inpatient	65	39.6	62	37.8	38.70[a] ± 1.03
Outpatient	20	12.2	17	10.4	11.28[b] ± 1.06
Mean% ± SD	25.90[a] ± 15.81		24.08[b] ± 15.84		

ANOVA

SOV	df	Mean Square	F- value	Sig.
Sex	1	6.625	6625	****
Dept.	1	1503.713	1503.713	****
Error	5	0.001		

**** P < 0.0001

Means with no common superscript are significantly different (p < 0.05) (SPSS, 2006)

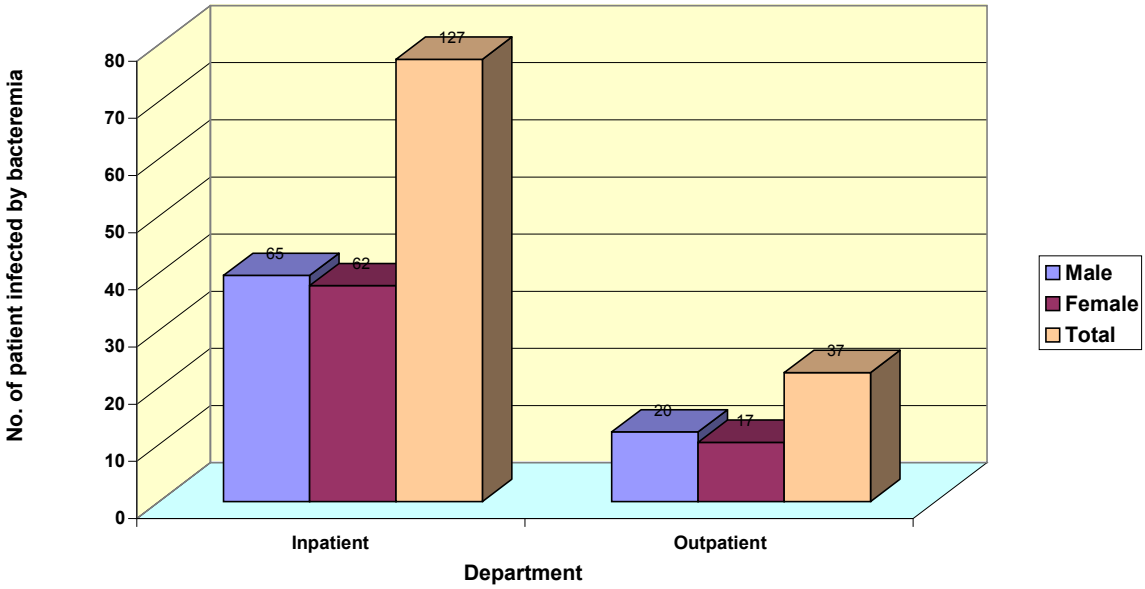

Fig. (2): Relationship between numbers of infected male and female and Departments

Relationship between Bacteremia and the Inpatient Departments

The statistical analysis of Bacteremia prevalence demonstrated that the patients in an Inpatient Departments showed that ICU registered the highest percentage of infection in as much as (35%), then the Peads Dept. (11%), then the EMD (9.7%), followed by surgery Dept. (6.1%), then Hemat/oncol Dept. (4.8%), Nephrology Dept. (RDU, HDU) (4.2%), Gastro-Enterology Dept. (1.8%), Urology Dept. and I D had the same percentage (1.2%) Intraverto Fertilization, L/D, Thoracic Dept. and Cardiology Dept. had the same percentage (0.6%) (Table 3, Fig. 3)

Table (3): Relationship between Bacteremia and Inpatient Departments

	Sex / Department	Male		Female		Mean% ± SD
		No.	%	No.	%	
Inpatient	Intensive Care Unit (ICU)	34	21	23	14	17.50^{a} ± 4.04
	Nephrology Department (Nephrology Dept.)	4	2.4	3	1.8	2.10^{de} ± .34
	Urology Department (Urology Dept.)	1	0.6	1	0.6	.60^{e} ± .00
	Pediatric Department (Peads Dept.)	13	8	5	3	5.47^{b} ± 2.80
	Surgery Department (Surgery Dept.)	3	1.8	7	4.3	3.05^{cd} ± 1.44
	Infection Disease Department (ID)	1	0.6	1	0.6	.60^{e} ± .00
	Hemato/oncology Department (Hemat/oncol Dept.)	3	1.8	5	3	2.42^{de} ± .72
	Emergency medicine Department (E M D)	4	2.4	12	7.3	4.85^{bc} ± 2.83
	Gastro-Enterology Department (Gastro-Enterology Dept.)	2	1.2	1	0.6	.90**e** ± .35
	Intravetro Fertilization (Intravetro Fertilization)	0	0	1	0.6	.30^{e} ± .35
	Labour/ Delivery (L/D)	0	0	1	0.6	.30^{e}± .34
	Cardiology Department (Cardiology Dept.)	0	0	1	0.6	.30^{e}± .34
	Thoracic Department (Thoracic Dept.)	0	0	1	0.6	.30^{e} ± .34
	Mean% ± SD	3.05 ± 5.67		2.90 ± 3.82		

ANOVA

SOV	df	Mean Square	F- value	Sig.
Sex	1	0.314	0.112	NS
Dept.	12	88.668	31.712	****
Error	38	2.796		

**** P < 0.0001 NS = Non Significant

Means with no common superscript are significantly different (p < 0.05) (SPSS, 2006)

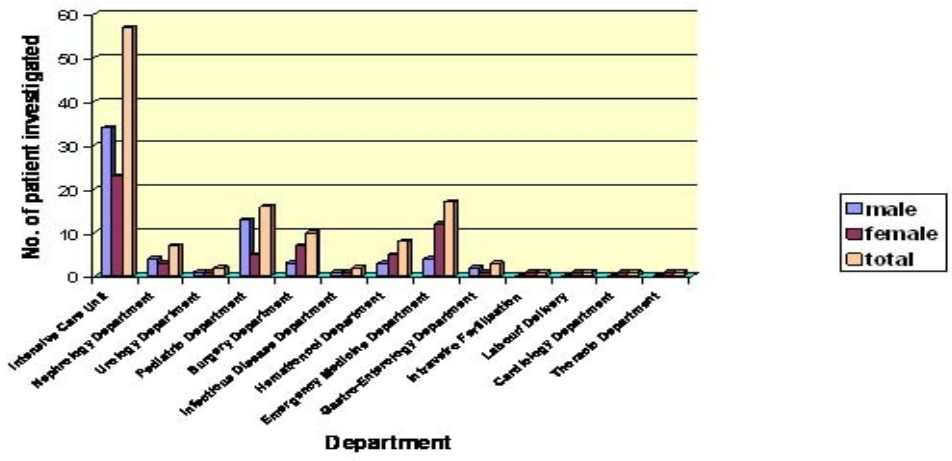

Fig. (3): The relationship between Bacteremia and numbers of infected males and females and the total number of each sex in an Inpatient departments

In an Outpatient Departments the DEM (Peads A/R) registered the highest percentage of infection

Relationship between Bacteremia and the Outpatients Departments

by bacteremia (20.8%), while PCC1, WBC and Pediatric Clinic had registered the same percentage (0.6%) (Table 4 and Fig. 4).

Table (4): Relationship between Bacteremia and Outpatients Departments

	Sex	Male		Female		Mean% ± SD
	Department	No	%	No	%	
Outpatient	DEM (Paeds A/R)*	18	11	16	9.8	10.35^{a} ± .635
	PCC1**	0	0	1	0.6	$.30^{b}$ ± .346
	WBC***	1	0.6	0	0	$.30^{b}$ ± .346
	Pediatric clinic	1	0.6	0	0	$.30^{b}$ ± .346
	Mean% ± SD	3.025 ± 4.867		2.600 ± 4.451		

* Department of Emergency Medicine (DEM), Pediatric Emergency Department (Absolute Risk) (Paeds A/R).
** Primary Care Clinic for Female (PCC1).
***Pediatric Clinic, Well Baby Care/Clinic (WBC).

ANOVA

SOV	df	Mean Square	F- value	Sig.
Sex	1	0.7225	4.744	NS
Dept.	3	101.0025	663.181	****
Error	11	0.1523		

**** P < 0.0001 NS = Non Significant
Means with no common superscript are significantly different (p < 0.05) (SPSS, 2006)

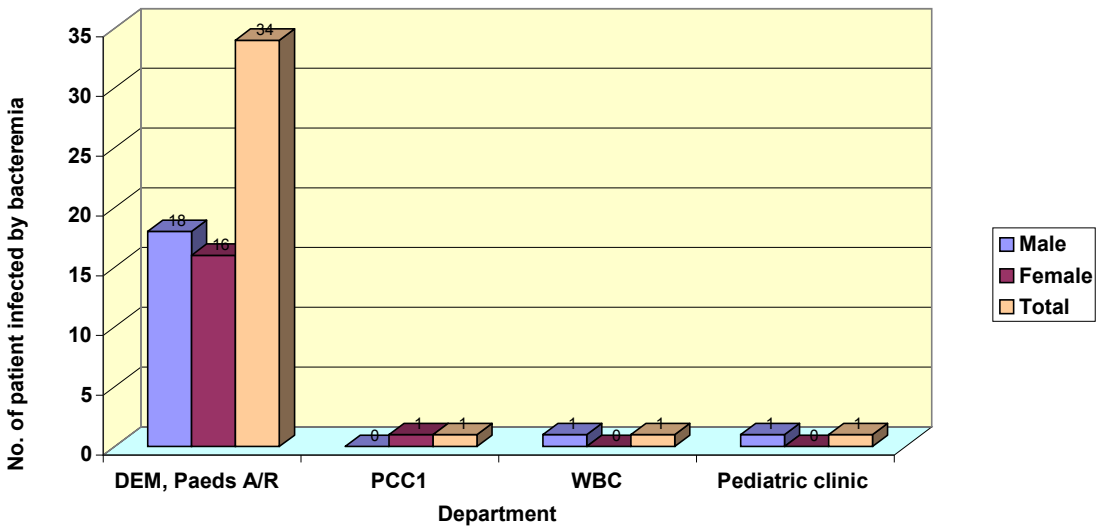

Fig. (4): The relationship between Bacteremia and numbers of infected Patients in an Outpatients Department

Relationship between Bacteremia and Isolated Microorganisms

The results illustrated that the patients can be infected by more than one microorganism causing bacteremia, in which there were 153 out of 164

cases (93.3%) infected by one bacterium, 8/164 cases (4.9%) were infected by more than one bacterium, and 3/164 cases (1.8%) were infected by yeast (Table 5).

Table (5): Numbers of cases infected by one or more than one microorganism

Microorganism	One bacterium		More than one bacterium		yeast		Total	
Infected cases	No.	%	No.	%	No.	%	No.	%
	153	93.3	8	4.9	3	1.8	164	100

Isolated Microorganism	One bacterium		More than one bacterium				yeast		Total	
			Two isolates		Three isolates					
	No.	%	No.	%	No.	%	No.	%	No.	%
	153	88.5	16	9.2	1	0.6	3	1.7	173	100

Relationship between Bacteremia with G⁻ve Bacteria and G⁺ve Bacteria

The statistical analysis in the Table below shows a non significant difference between the infected patients by either G⁻ve or G⁺ve in both males and females (Table 6).

Table (6): Relationship between bacteremia and Gram Negative bacteria and Gram Positive bacteria

Bacteria / Sex	G⁺ve	G⁻ve	Mean% ± SD
Male	49	36	43.00 ± 8.08
Female	38	47	42.50 ± 6.35
Mean% ± SD	43.50 ± 7.50	42.00 ± 6.92	

ANOVA

SOV	df	Mean Square	F- value	Sig.
Kinds of bacteria	1	4.500	0.014	NS
Sex	1	0.500	.713	NS
Error	5	312.50		

NS = Non significant (SPSS, 2006)

Genera of Gram Negative Bacteria Causing Bacteremia

The Table below illustrates that there were significant differences ($p<0.0001$) between genera of G⁻ve bacteria which infected patients, where *E. coli* exhibited significant differences in comparison with other genera isolated from blood, while there were no significant differences between other tested genera. The statistical analysis showed that there was a non significant difference between the percentage of infection by G⁻ve bacteria in males and females (Table 7 and Figures 5 and 6).

Table (7): Genus of Gram Negative Bacteria Causing Bacteremia

Bacteria \ Sex		Male		Female		Mean% ± SD
Genus of G-ve	Species	No. of Isolates	%	No. of Isolates	%	
Escherichia	*E. coli*	4	4.8	15	17.9	11.35^{a} ± 7.56
Pseudomonas	*P. aeruginosa*	8	9.5	5	5.9	7.70^{b} ± 2.07
Klebsiella	*K. pneumoniae*	5	5.9	4	4.8	5.35^{bc} ± .635
Proteus	*Pro. mirabilis*	0	0	1	1.2	$.60^{d}$ ± .69
Acinetobacter	*A. lwoffi* *A. baumannii/haemolyticus*	6	7.1	6	7.1	7.10^{b} ± .00
Enterobacter	*En*. agglomerans *En. cloacae*	1	1.2	3	3.6	2.40^{cd} ± 1.38
Citrobacter	*Citro. koseri* *Citro. freundii*	2	2.4	0	0	1.20^{d} ± 1.38
Alcalignes	*Alca. xylosoxidans* subsp*. xylosoxidans* *Alcalignes* sp.	0	0	2	2.4	1.20^{d} ± 1.38
Haemophilus	*Haem. influenza*	0	0	1	1.2	$.60^{d}$ ± .692
Moraxella	*M. catarrhalis*	1	1.2	0	0	$.60^{d}$ ±.692
Achromobacter	*Ach. xylosoxidans* subs *xylosoxidans*	2	2.4	1	1.2	1.80^{d} ± .69
Brucella	*Brucella* sp.	2	2.4	6	7.1	4.75^{c} ± 2.71
Salmonella	*Salmonella* sp. *Salmonella* sero group D1	5	5.9	1	1.2	3.55^{cd} ± 2.71
Ochrobactrum	*Ochro. anthropi*	0	0	1	1.2	$.60^{d}$ ± .692
Serratia	*Ser. marcescens*	1	1.2	1	1.2	1.20^{d} ± 00
Mean% ± SD		2.93 ± 2.94		3.73 ± 4.52		

ANOVA

SOV	df	Mean Square	F- value	Sig.
Sex	1	9.6	1.725	NS
Genus	14	42.972	7.723	****
Error	44	5.564		

**** P < 0.0001 NS = Non Significant

Means with no common superscript are significantly different (p < 0.05) (SPSS, 2006)

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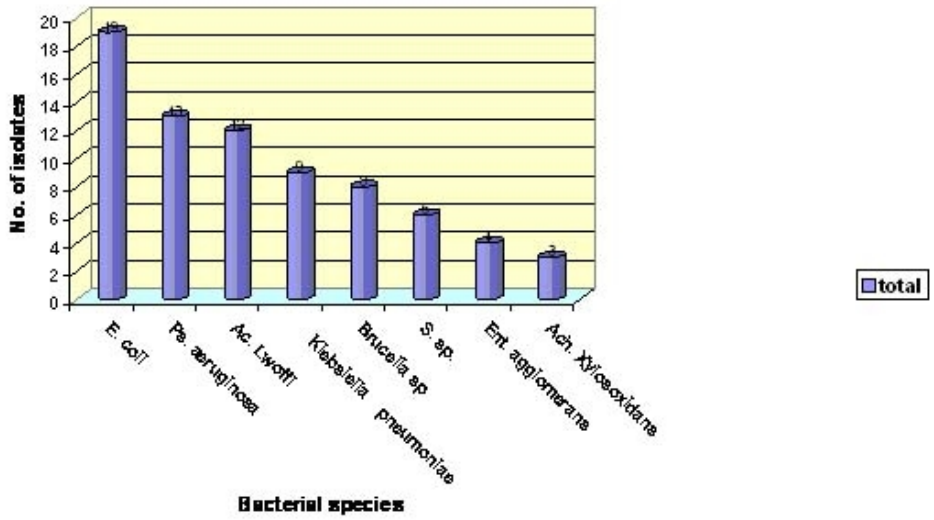

Fig. 5: Number of isolated Gram Negative Bacteria of patients which are the most frequency causing bacteremia

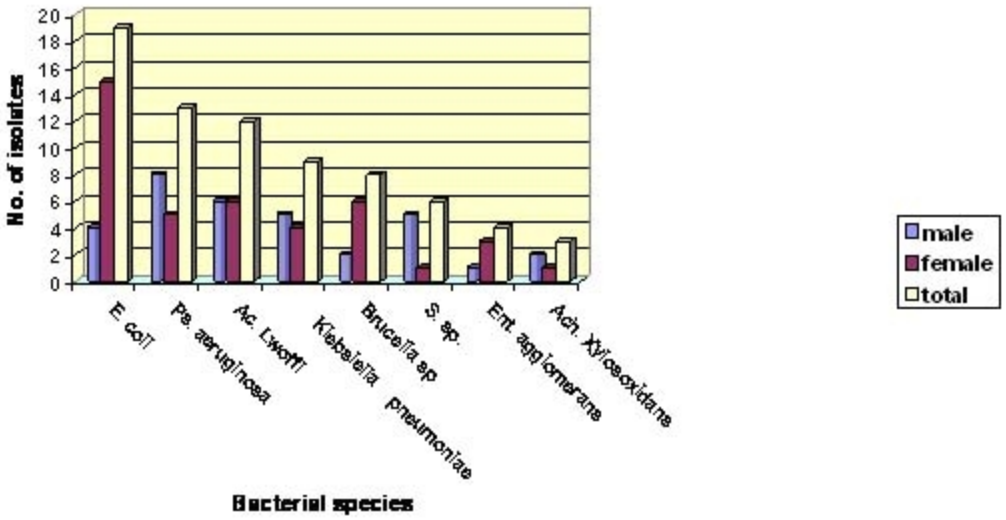

Fig. 6: The relationship between numbers of isolated Gram Negative Bacteria of patients which are the most frequency causing bacteremia and number of infected males and females and their total.

Genera of Gram Positive Bacteria Causing Bacteremia

The Table below illustrates that there were significant differences ($p<0.0001$) between genera of G^{+}ve bacteria which infected patients, where *Staphylococcus* sp. exhibited significant differences in comparison with other genera isolated from

blood, while there were no significant differences between other tested genera. The statistical analysis showed that there was a non significant difference between the percentages of infection by G+ve bacteria in males and females (Table 8, Figures 7 and 8).

Table (8): Genus of Gram positive bacteria (G+ve) causing bacteremia

Bacteria \ Sex		Male		Female		Mean% ± SD
Genus of G+ve	Species	No. of Isolates	%	No. of Isolates	%	
Streptococcus	*S. pneumoniae* *S. salivarius* *Streptococcus* Group A *S. viridans* *S. Mitis* *S. sanguis* *Streptococcus* Group D *Streptococcus* Group B	6	6.9	7	8	7.45^{b} ± .63
Staphylococcus	*Staph. aureus* *Staph. simulans* *Staph. hominis* subsp. *hominis* *Staph. haemolyticus* *Staph. epidermidis* *Staph. auricularis* *Staph. sciuri* *Staph. xylosus* *Staph. Capitis* subspecies *ureolyticus* *Staph. hominis* subsp. *novobiosepticus* MRSA	31	35.6	24	27.5	31.55^{a} ± 4.67
Enterococcus	*Entero. faec*alis	4	4.6	0	0	2.30^{c} ± 2.65
Bacillus	*Bacillus* sp.	5	5.7	3	3.5	4.60^{bc} ± 1.27
Diphtheroids	*Diphtheroids* sp.	3	3.5	3	3.5	3.50^{c} ±.00
Micrococcus	*Micrococcus* sp.	0	0	1	1.2	0.60^{c} ± .69
Mean% ± SD		9.38 ± 12.45		7.28 ± 9.79		

ANOVA

SOV	df	Mean Square	F- value	Sig.
Sex	1	26.46	6.6355	NS
Genus	5	538.639	135.065	****
Error	17	3.988		

**** P < 0.0001 NS = Non Significant

Means with no common superscript are significantly different (p < 0.05) (SPSS, 2006)

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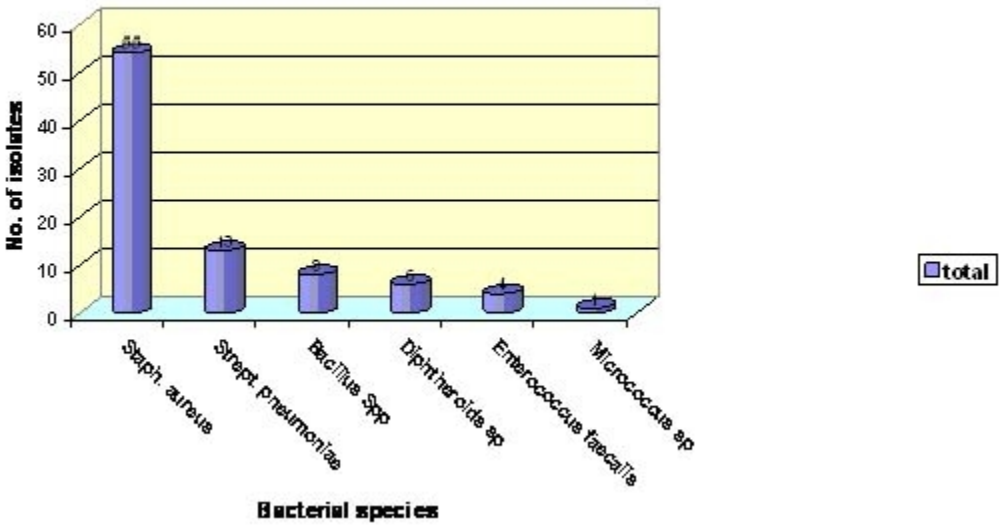

Fig. (7): Number of isolated Gram Positive Bacteria of patients which are the most frequency causing bacteremia

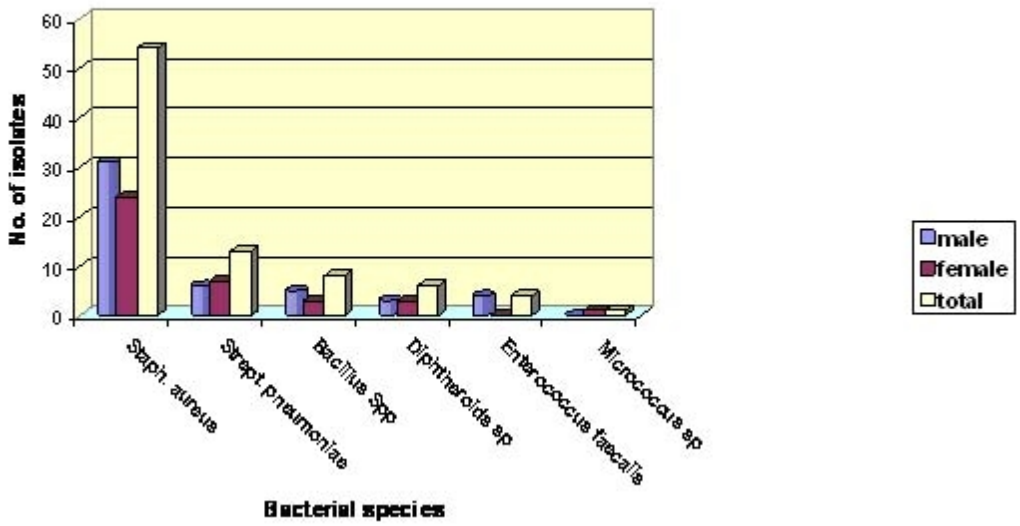

Fig. (8): The relationship between numbers of isolated Gram Positive Bacteria of patients which are the most frequency causing bacteremia and number of infected males and females and their total.

DISCUSSIONS

The study discussed the relationship between bacteremia and age, which illustrated that the percentage of elderly was the highest (38.3%), compared to the adults category (33.2%), then came the other categories (infants, pediatrics, teenagers) which were statistically equal, (13.2, 11, 4.3%) respectively. The prevalent bacteria which were associated with bacteremia were *Staphylococcus* sp. as G^+ve bacteria and *E. coli* as G^- ve bacteria, which disagreed with Frederiksen *et al*., (2007) who reported that the incidence of bacteremia in an infant age group had greater percentage (73.9%) compared with other age strata, and supports that *Staph. aureus* is dominant bacteria in bloodstream.

Results obtained in our study showed that the highest percentage of infections was in an Intensive Care Unit (35%) for both genders, which was opposed Juanjuan *et al*., (2007) who noted that the highest percentage of infections was in the Department of Urological Surgery (48.1%).
Results from the previous studies (Gray *et al*., 2001 and Juanjuan *et al*., 2007) were consistent with the present result, which revealed that the percentage of patients in an Inpatients Departments (77.4%) was highly significant than the percentage of infected patients in an Outpatients Departments (22.6%). The present results supported Rahbar *et al.*, (2005) who found that the percentage of G^+ve and G^-ve bacteria causing bacteremia were equal, while, Espinosa *et al*., (1999) revealed that G^-ve bacteria were more than G^+ve bacteria.

It was worth mentioning, according to the cultural, morphological and physiological characters of the isolated bacteria from bloodstream, that 8 patients were infected by more than one genus of bacterium (Gupta, *et al.,* 2005), 153 patients were infected by one genus of bacterium and 3 patients were infected by yeast. According to the available literatures, it seems that infection by more than one genus of bacteria was considered as one of the first record in Saudi Arabia. To follow the differences in etiology and outcome of bacteremia in Riyadh, city, Saudi Arabia, a study at Security Forces Hospital, for Saudi's patients only, was carried out after 2 years from our study (Alsayed, M. F. S., 2010). Results obtained from 50 samples of blood were collected from Inpatients and Outpatients departments, seems to be close to our findings this means that overview of bacterimia within 2 years didn't change either in Saudi population or Saudi with foreign population in Riyadh City.

CONCLUSIONS

Out of 164 samples, 20 species belonging to 15 G^-ve genera and 23species belonging to 6 G^+ve genera were isolated from blood stream infection.
Statistical analysis illustrated the following: a significant effect in the age categories, infected people in an Inpatient Departments was higher incorporeal than infected people in an Outpatient Departments. ICU and DEM (Peads A/R) registered the highest percentage of infection in an Inpatient and Outpatient Departments respectively.
The results illustrated that the patients can be infected by one bacterium, more than one bacterium or yeast.
E. coli and *Staphylococcus* sp. exhibited significant differences in comparison with other genera (G^-ve and G^+ve respectively) isolated from blood, while there were no significant differences between other tested genera. The statistical analysis showed a non significant difference between the infected patient by either G^-ve or G^+ve in both males and females.

ACKNOWLEDGEMENT

Thanks for King Abdulaziz City for Science and Technology (KACST)) Contribution No. AT-18-75) and the Research Center in the Departments of Science and Medical Studies in King Saud University for its Grant to support this research project.

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How to Cite this Article: Roua M. S. Alkufeidy, Alia A. Shoeib, Ali M. Somily, "A Study of Epidemiology and Etiology of Bacteremia Isolates from Patients in Riyadh City of Saudi Arabia " , Science Journal of Microbiology, Volume 2012, Article ID sjmb-112, 13 Pages, 2012. doi: 10.7237/sjmb/112