Research Article

Blood Cultures Positive for CoNS- and Antibiotics Sensitivity Patterns in Al-Assad University Hospital

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ABSTRACT

Coagulase-negative staphylococci (CoNS), is the most common nosocomial bloodstream infection, inspite it was considered as skin flora. In the first of May 2011, at the department of microbiology of Al-Assad university hospital, the research was conducted and lasted for one year. The study sample was 50CoNS positive blood cultures. Among 50-CoNS isolates, *Staphylococcus epidermidis* was the most prevalent species: 22 samples, followed by *Staphylococcus haemolyticus*: 11 samples, *Staphylococcus hominis*: 6 samples, *Staphylococcus xylosus*: 5 samples, *Staphylococcus warneri*: 3 samples, *Staphylococcus saprophyticus*: 2 samples, *Staphylococcus cohnii*: 1 sample. Resistance to methicillin was detected among 68% of CoNS isolates. Methicillin-resistant CoNS strains were determined to be more resistant to antibiotics than methicillin-susceptible CoNS strains except tetracycline, chloramphenicol, fusidic acid. None of the strains were resistant to vancomycin and teicoplanin and linezolid, except hyposensitivity in some cases for teicoplanin. The incidence of significant CoNS bacteremia was 10 of 50 (20%), that of indeterminate bacteremia was 7 of 50 (14%), and that of contamination was 33 of 50 (66%).

Keywords: Blood cultures, coagulase negative staphylococci, blood stream infection, antibiogram tests.

INTRODUCTION

Albeit staphylococci are present among normal human flora of skin and mucosal barriers; Coagulase-negative staphylococci blood infections are the most frequent in hospital related blood infections and stem from skin and mucosal barriers[1] essentially at the catheters' entry "catheters' related blood infections".[2]

According to National Nosocomial Infections Surveillance system NNIS, CoNS blood infections present between 1992 and 2001 reached 36% of blood infections and 72% of newborn blood infections. [3]

Nosocomial infections[4]

Infections got after hospitalization and their manifestations appear in a delay superior to 48 hours because of two factors: 1) patient related factors including disease intensity, immune depression and long hospitalization, 2) hospital milieu related factors including medical staff, visitors, other patients and medical equipment. Those factors predispose patient to be invaded by normal flora saprophytes changed into pathogens.

According to NNIS the most hospital related infections are urinary infections 36%, surgical wounds infections 20%, and pneumonia 11% and primary blood infections 11%. These infections lay behind high morbidity and high mortality among hospital patients. On the authority of CDC in 2002, it was registered in USA 1.7 million cases of nosocomial infections resulted in 99000 mortality. [5]

Pathologic mechanism

CoNS strains are the most frequent human skin bacterial saprophytes. They secrete an exo-polysaccharide named "Slime" which participate in bacterial adherence on polymer foreign surfaces (like medical prosthetics) and in a biofilm formation that increases bacterial clumping and renders them more resistant to antibiotics and phagocytosis. Owing to this immune modifying effect, opportunistic infections by such bacteria increase in patients with central catheters, peripheral catheters, and medical prosthetic or vascular bypass. Frequency rate increases also in intensive care unit, incubators and hemodialysis sections and in cancer patients as well.[6]

MATERIALS AND METHODS

Fifty blood culture samples from different hospital sections fulfilled CoNS positive diagnosis. This study was exempted from age, sex, and certain hospital section.

Diagnostic processes applied to samples

- Blood samples were automatically cultured by incubating blood culture bottles in automatic Bactec system.[7]
- Positive samples were transferred to blood agar plates; and standard ways were proceeded to recognize microbe (Catalase test, coagulase test, and gram staining)[8].
- Biochemical typing in order to determine CoNS strains. This step was done by using CoNS strains biochemical typing kits (API-Staph) bio Mearieux – France[9]. Definition system consists of a band with

19 wells which contains 19 biochemical tests detecting bacterial ability to ferment some saccharides and to produce some enzymes, and its reduction power. The tests above permit staphylococci strains differentiation.

- Antibiogram by disk-diffusion test[10] was the way we relied on in our study. It's achieved by microbe isolation: surface plate microbe diffusion and sensitivity, the plates were incubated at 35° - 37° temperature for 18 – 24 hours then results reading take place:
  1. In close disc proximity microbial growth means that antibiotic doesn't influence the microbe which is considered resistant (R)
  2. Around disc transparent halo formation with variable diameters proportional to antibiotic activity means that antibiotic influence the microbe which is considered sensitive (S)
  3. Tested antibiotics were: Vancomycin, Teicoplanin, Gentamycin, Tetracycline, Chloramphenicol, Rifampicin, Clindamycin, Oxacillin, Fusidic acid, Cephalothin, Trimethoprim-Sulfamethoxazole (Bactrim), Cefuroxime, Imipenem, Linezolid.

RESULTS AND DISCUSSION

Statistical analysis

Statistical analysis was accomplished by assistance of STATA statistical program and by using the following statistical tests:

- Kai square test in view to study interrelation between separated variables, and Fisher's exact test when Kai square test praxis conditions are unfit.
- Differences at designation threshold ≤ 0.05 were considered statistically significant.

Through one year (1 May – 30 April) 50 confirmed positive CoNS blood culture samples -sent by Al-Assad University Hospital departments to microbiology section- were studied. 7 CoNS strain types were isolated by biochemical typing; the following table -Table (1)- show their distribution.

Table 1: demonstrate CoNS-Epidermidis was the most frequent 44% (22 case in 50 samples), followed respectively by CoNS-Haemolyticus 22%, then CoNS-Hominis 12%, then CoNS-Xylosus 10%, and CoNS-Chonii was the least common strain 2%.

Frequency rate of CoNS positive blood cultures

Frequency rate of CoNS positive blood cultures compared to other agents reached 30% (50/170), table (2) shows CoNS positive blood cultures frequency in comparison with other pathogenic agents through study period.

Table 1: CoNS Strains Distribution

CoNS	Frequency	Percentage
Epidermidis	22	44
Haemolyticus	11	22
Hominis	6	12
Xylosus	5	10
Warneri	3	6
Saprophyticus	2	4
Chonii	1	2
Total	50	100

Table 2: CoNS positive blood cultures frequency in comparison with other pathogenic agents through study period

positive blood cultures	Frequency	Percentage
CoNS positive blood cultures	50	29.4%
other positive blood cultures	120	70.6%
Total	170	100%

Types of antibiotics sensitivity and resistance:

- Methicillin and/or Oxacillin resistance appeared in 68% of cases
- The lowest resistance was to Rifampicin 4%, and the highest one was to Bactrim 70%
- The proportion of Methicillin-resistant strains which resist other antibiotics is superior to Methicillin-sensitive strains. Chloramphenicol, Tetracycline and Fusidic acid are exceptions to that rule.
- Either Vancomycin or Linezolid resistance wasn't registered, but Teicoplanin hyposensitivity was observed in some cases.
- Some strains showed Teicoplanin hyposensitivity manifested by around disc diameter reduction. These accidents may be ascertained by more reference methods; such as E-test or dilution methods in order to define MIC (minimum inhibitory concentration) which determine exactly the effective concentration on microbe and if this effect is bactericide or bacteriostatic.

Table (3) reveals incubators section has the greatest ratio of CoNS positive blood cultures 20/50. Pediatric section comes next 14/50, whereas surgical section and cardiac catheterism have the least ratio 2/50 each.

Established positive CoNS blood cultures distribution study in function of hospital sections

Table 3: Established positive CoNS blood cultures distribution study in function of hospital sections

Source of blood culture samples		Staph. Positive Blood Cultures			
		CoNS	Others	Total	*P*-Value
Pediatric Section	frequency	**14**	**43**	**57**	0.26
	percentage	24.56	75.44	100	
Incubators Section	frequency	**20**	**38**	**58**	
	percentage	34.48	65.52	100	
ICU	frequency	**4**	**14**	**18**	
	percentage	22.22	77.78	100	
Hemodialysis	frequency	**4**	**2**	**6**	
	percentage	66.67	33.33	100	
Internal Section	frequency	**4**	**17**	**21**	
	percentage	19.05	80.95	100	
Surgical Section	frequency	**2**	**3**	**5**	
	percentage	40	60	100	
Cardiac Catheterism	frequency	**2**	**3**	**5**	
	percentage	40	60	100	
Total	frequency	**50**	**120**	**170**	
	percentage	29.41	70.59	100	

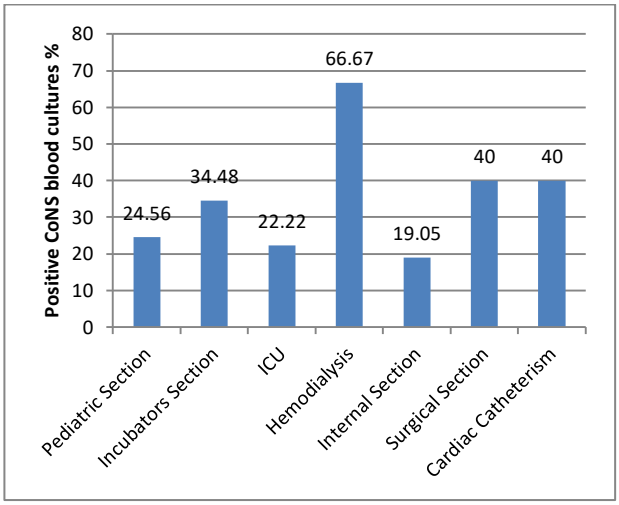

Figure 2: CoNS positive blood cultures frequency rate in different hospital sections in comparison with other pathogenic agents

Fig(2): reveals the highest frequency rate of CoNS positive blood cultures -in comparison with other pathogenic agents- was seen in samples issued from hemodialysis 67% (4/6), whereas the lowest one was seen in internal section approximately 20% (4/21). There is a difference in CoNS positive blood cultures frequency rate between samples sent from different hospital sections, but this difference is worthless from statistical standpoint (statistical significance degree > 5) as evidenced in table 3.

Rates of CoNS resistance for antibiotics

CoNS resistance for Gentamycin overall percentage reached 42%. It was variable from one strain to another (ranged from 0% in CoNS.*chonii* to 100% in CoNS.*saprophyticus*, and reached 13.6% in CoNS.*epidermidis*) and was statistical important (degree of statistical importance was 0.002).and so are the antibiotics (Cefuroxime, Imipenem, Clindamycin, Cephalothin) where degree of statistical importance was inferior to 0.05.

Methicillin-resistance[11]

CoNS Oxacillin resistance reached 68% with no statistically important difference between the strains (degree of statistical importance=0.7). Fig(3) shows CoNS overall resistance percentage to all of the eleven tested antibiotics. We Observe that the resistance for Bactrim (SXT) is the highest one 70%, followed by resistance for Oxacillin (OX) 68%, whereas the resistance for Chloramphenicol (C) and Rifampicin (RA) is the lowest 6% and 4% respectively.

CoNS cross resistance with Methicillin

Table (5) shows the relation between CoNS resistance for Methicillin V.S its resistance for other antibiotics. We observe that Methicillin-resistant CoNSare -statistically important- more resistant for the following antibiotics

than Methicillin-sensitive CoNS: KF (38.24% V.S 6.25% degree of statistical importance 0.05), CXM (50% V.S 11.76% degree of statistical importance 0.01), DA (32.35% V.S 0% degree of statistical importance 0.007), IPM (47.26% V.S 6.25% degree of statistical importance 0.05), SXT (85.29% V.S 58.82% degree of statistical importance 0.03); while there are no statistically important differences between existence of resistance for Methicillin and existence of resistance for other antibiotics.

Table (4): CoNS strains resistance for antibiotics

CoNS Strains		Studied Antibiotics										
		CN	KF	OX	SXT	CXM	FA	DA	TE	IPM	C	RA
chonii	**Freq.**	0	0	0	0	1	0	0	0	0	0	0
	R-per.	**0**	**0**	**0**	**0**	**100**	**0**	**0**	**0**	**0**	**0**	**0**
epidermidis	**Freq.**	3	1	14	17	4	10	1	2	1	1	0
	R-per.	**9.1**	**0**	**59.1**	**68.2**	**13.6**	**45.5**	**4.6**	**9.1**	**0**	**0**	**0**
haemolyticus	**Freq.**	7	7	9	7	7	2	2	0	0	1	0
	R-per.	**63.6**	**63.6**	**81.8**	**63.6**	**63.6**	**18.2**	**18.2**	**0**	**0**	**9.1**	**0**
hominis	**Freq.**	3	1	4	4	2	3	2	3	4	0	2
	R-per.	**50.0**	**16.7**	**66.7**	**66.7**	**33.3**	**50.0**	**33.3**	**50**	**66.7**	**0**	**33.3**
saprophyticus	**Freq.**	2	0	2	2	2	0	2	0	2	0	0
	R-per.	**100**	**0**	**100**	**100**	**100**	**0**	**100**	**0**	**100**	**0**	**0**
warneri	**Freq.**	2	0	2	1	0	1	2	1	0	0	0
	R-per.	**66.7**	**0.0**	**66.7**	**33.3**	**0.0**	**33.3**	**66.7**	**33.3**	**0**	**0**	**0**
xylosus	**Freq.**	4	3	3	4	3	3	2	1	3	1	0
	R-per.	**80**	**60**	**60**	**80**	**60**	**60**	**40**	**20**	**0**	**20**	**0**
Total	**Freq.**	21	12	34	35	19	19	11	7	10	3	2
	R-per.	**42**	**24**	**68**	**70**	**38**	**38**	**22**	**14**	**20**	**6**	**4**
p value		**<0.002**	**<0.001**	0.7	0.45	**<0.01**	0.51	**<0.005**	0.08	**<0.001**	0.59	0.08

(CN)Gentamycin, (TE)Tetracycline, (C)Chloramphenicol, (RA)Rifampicin, (DA)Clindamycin, (OX)Oxacillin, (FA)Fusidic acid, (KF)Cephalothin, (SXT)Trimethoprim-Sulfamethoxazole, (CXM)Cefuroxime, (IPM)Imipenem. // Freq.= Frequency, R-per= resistance percentage //

Table (4) shows resistance types of CoNS strains to all studied antibiotics:

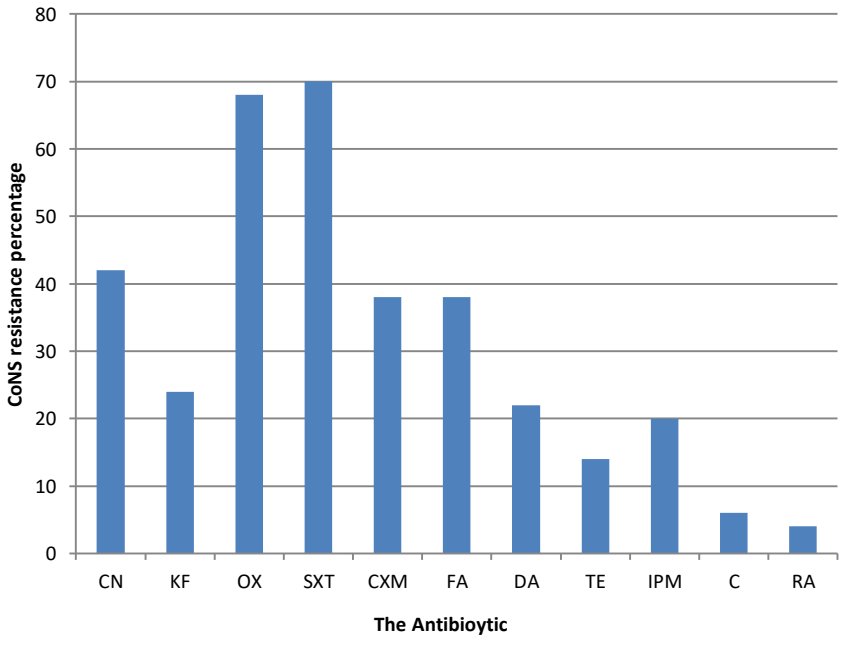

Figure 3: Type of CoNS resistance for antibiotics

Table 5: Methicillin Sensitivity

		MS	MR	*P.* value
CN	Frequency	4	17	0.09
	Cross.R per. *	23.53	50.00	
KF	Frequency	1	13	**0.05**
	Cross.R per.	6.25	38.24	
SXT	Frequency	9	29	**0.03**
	Cross.R per.	58.82	85.29	
CXM	Frequency	2	17	**0.01**
	Cross.R per.	11.76	50.00	
FA	Frequency	7	11	0.34
	Cross.R per.	47.06	33.33	
DA	Frequency	0	11	**0.007**
	Cross.R per.	0	32.35	
TE	Frequency	4	3	0.16
	Cross.R per.	23.53	9.09	
IMP	Frequency	1	9	**0.05**
	Cross.R per.	6.25	47.26	
C	Frequency	1	2	0.63
	Cross.R per.	6.25	5.88	
RA	Frequency	0	2	0.3
	Cross.R per.	0	6.06	

*Cross.R per.= Cross resistance percentage

Bacteraemia Classification

Depending on international studies, we classified bacteraemia into True-CoNS bacteraemia and False-CoNS bacteraemia (contamination)[12]:

- Clinical features: fever over 38°, Hypotension < 9 mm/Hg, and intravascular disseminated coagulation. Those criteria are favor True-CoNS bacteraemia[13]
- Laboratory features: neutrophilic leukocytosis with left shifted formula, CRP elevation. Thos criteria are also favor True-CoNS bacteraemia.
- Microbiologic features favor True-CoNS bacteraemia:
  1. Time required for positive blood culture: positivity during the first 72 hours approves True-CoNS bacteraemia.
  2. Number of positive blood cultures: > 2 positive blood cultures approves True-CoNS bacteraemia.

The adopted criteria for newborns and younger infants are different from those for older infants and adults. Features support True-CoNS bacteraemia in newborns and younger infants include: apnea and increased necessity for respiratory support, tachypnea, temperature instability, abdominal distention, nutrition troubles, metabolic acidosis, WBC variations (increase or decrease), platelets decrease, and hyperglycemia. [14]

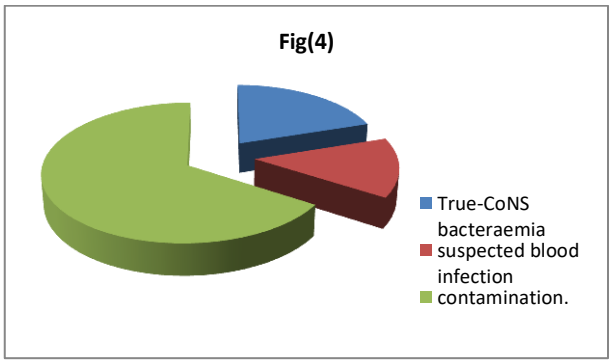

Figure 4: shows 50 cases partition:

10/50 (20%) were distinguished as True-CoNS bacteraemia, 7/50 (14%) suspected blood infection, whereas 33/50 (66%) were classified as contamination.

CONCLUSIONS

- Through a year-long study (2011-2012) in Al-Assad University Hospital, CoNS positive blood culture reached 30% and *Staph*. *epidermidis* was the most frequent strain.
- Strains showing highest microbial resistance are important to be determined in order to outline the

appropriate antibiotics for their treatment. *Staph. saprophyticus* was the most resistant strain for tested antibiotics.

- CoNS strains resistance for Methicillin reached 68%, and the highest resistance percentage was for Trimethoprim (70%).
- No resistance emerged for Vancomycin or Linezolid, whereas Teicoplanin hyposensitivity was observed in some cases, which considered as warning alarm of pre-resistance for glycopeptides antibiotics.
- All patients showing CoNS positive blood cultures were previously treated by antibiotics (beta-lactams and amino glycosides particularly), but CoNS strains -statistically important-were resistant for these antibiotic according to our study. In consequence of high percentage of Methicillin-resistant CoNS, beta-lactams shouldn't be used in treatment of CoNS strains. [15]
- Vancomycin has been used to treat eight cases with true-CoNS bacteraemia, eleven cases with contamination and two cases of suspected blood infection. We stress here on not misusing glycopeptides antibiotics because they are the last existing refuge in treatment of multi-antibiotics resistant CoNS. One of the cultures classified among True-CoNS bacteraemia exhibited only one time positive blood culture but associated diagnostic clinical and laboratory features, while the rests exhibited two or three times positive blood cultures. All the contaminated cases didn't exhibit more than one time.
- Despite CoNS high sensitivity for Rifampicin, this medication was rarely used in these infections. Vancomycin-Rifampicin synergism is considered a successful treatment in deep situated CoNS infections (prosthetic endocarditis, CNS-Vascular bypass infections)
- Hospital conduct in antibiotics using policy plays its role in CoNS-Resistant strains selection. Methicillin and Trimethoprim resistance was -in our study-the most frequent among CoNS strains.
- The majority of patients had peripheral venous catheters, so it was difficult to study the effect of existence or absence in reduction of blood cultures contamination.

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