JOURNAL OF CURRENT RESEARCH IN SCIENCE (ISSN 2322-5009)
CODEN (USA): JCRSDJ
2013, Vol. 1, No. 6, pp:489-494
Available at www.jcrs010.com

ORIGINAL ARTICLE

COMPARATIVE ANALYSIS OF MULTI-DRUG RESISTANCE PATTERN IN *STAPHYLOCOCCUS AUREUS* ISOLATED FROM SKIN OF VARIOUS PROFESSIONAL GROUPS

Md. Afzal Hossain[1,2], Hafsa Afroz[1], Md. Abdur Rahim Khan[1,3], Md. Manjur Hossain[1,4], A.S.M. Ruhul Quddus[1,5], Bidhan Chandra Sengupta[1,6], Md. Fakruddin[7], Zahed Uddin Mahmood Khan[1] and Suvamoy Datta[1]

1- Department of Microbiology, Primeasia University, Dhaka-1213, Bangladesh
2- Department of Microbiology, Z.H. Sikder Woman's Medical College, Dhaka, Bangladesh
3- Essence Homoeopathy Medical Centre, 7/A, Shobhanbag, Sher-e-Bangla Nagar, Dhaka, Bangladesh
4- Medical Officer (MCH-FP), Bhaluka Upazila Health Complex, Bhaluka, Mymensingh, Dhaka, Bangladesh
5- Department of Pathology, Nightingale Medical College, Dhaka, Bangladesh
6- Department of Clinical Pathology, Chittagong Medical College Hospital, Chittagong, Bangladesh
7- Institute of Food Science and Technology (IFST), Bangladesh Council of Scientific and Industrial Research (BCSIR), Dhaka, Bangladesh

ABSTRACT: *Staphylococcus aureus* is a recognized human pathogen responsible for a great variety of pyogenic infection in man and animals. The aim of this study was to determine prevalence and antibiotic resistance pattern of Staphylococcus aureus isolated from skin of different professional groups. A total of forty (40) Staphylococcus aureus strains were isolated from skin of 4 professional groups: teachers, students, patient samples, and nurses. The strains were identified on the basis of biochemical tests to be *Staphylococcus aureus*. Agar disc diffusion test was performed to determine antibiotic susceptibility pattern of the isolates. Nurses and Patient samples exhibited a higher resistance rate than normal microflora isolates from teachers and students. One of the nurse samples was resistant to at least 9 antibiotics, and one of the patient samples was resistant to at least 8 antibiotics. Over 62.5% of the strains are resistant to 5 or more of the antibiotics tested. 100% of the isolates tested were susceptible to vancomycin, where 95.0%, 92.5% and 73.5% of the isolates tested were susceptible to bacitracin, ciprofloxacin and cefaclor respectively.

KEYWORDS: *Staphylococcus*, Skin, Professional, Antibiotic Resistance.

INTRODUCTION

Staphylococcus aureus occurs as a commensal on human skin, particularly the scalp, armpits, and nasopharynx; its primary habitat is the moist squamous epithelium of the anterior nares (Foster, 2004). *S. aureus* is commonly carried on the skin or in the nose of healthy people. *S. aureus* also occurs widely on environmental surfaces and is particularly of concern in hospital environments because of its presence in bed sheets, clothing and other fomites (Von Eiff and Becker, 2001; Le Loir *et al.*, 2003). *S. aureus* is an important pathogen due to a combination of toxin-mediated virulence, invasiveness and antibiotic resistance (Shopsin and Kreiswirth, 2001). Over the past several decades, it has emerged as one of the most important human pathogens and has become a leading cause of nosocomial infections (Lowy, 1998). It may cause a variety of clinical infections with high morbidity and mortality rates; these include wound sepsis, abscess formation, septicemia, pneumonia, osteomyelitis, post-surgical infection, toxic shock syndrome and septic arthritis (Boyce, 1997; Shopsin and Kreiswirth, 2001; Cosgrove *et al.*, 2003).

Antibiotic resistance can cause serious disease and is an important public health problem. In many developing countries like Bangladesh, over-the-counter sale of antibiotics is widespread and this accelerates resistance among the common pathogens (Olufemi *et al.*, 2011). Unnecessary prescriptions, e.g. even in the case of viral illnesses in which case antibiotics are ineffectual, are also a contributing factor (Coplan *et al.*, 2003). In addition, antibiotics are routinely used as growth promoters in farm animal feeds, for prophylaxis and in feed additives in aquaculture (Cabello, 2006). The staphylococci are known to be variably resistant to many antibiotics (Kluytmans and Mouton, 1995). They are

Corresponding Author: Suvamoy Datta, Associate Professor and Head Department of Microbiology, Primeasia University, 9 Banani, Dhaka-1213, Bangladesh. **E-mail:** suvamoy.datta@primeasia.edu.bd

currently resistant to almost all of the older antibiotics; this crisis has been fueled by the extensive use of antibiotics in the community and hospitals (Neu, 1991).

A comparative analysis of the antibiotic resistance patterns of *S. aureus* isolated from skin of various professional groups was carried out in order to evaluate the current status of antibiotic resistance pattern in this organism.

MATERIALS AND METHODS

2.1. Collection of Samples

Specimens were collected from skin of different professional groups (teachers, students, patient samples and nurses). A total of 40 specimens were collected; 10 samples were obtained from each category. Sterile moistened cotton swabs were used to obtain bacteria from skin surfaces such as those between the toes and behind the ears.

2.2. Isolation of strains

All swab samples were transported to the laboratory within 24 hours after collection. The specimens were processed in the bacteriology section of the microbiology laboratory. The organisms were enriched by overnight incubation in test tubes of sterile nutrient broth and then streaked over the surface of MSA plates and incubated at 37°C for 18 to 24 h. After incubation, single golden-yellow colonies suspected to be *Staphylococcus aureus* were selected from each of the plates and subcultured onto nutrient agar for pure culture and for presumptive identification. *Staphylococcus aureus* strain ATCC 29213 was used as reference strain.

2.3. Identification of Staphylococcus aureus strains

The shape and type of Gram reaction are microscopically studied using 18 hour culture from agar plate. The biochemical tests involved are Kliger's Iron (KIA) Agar, Simmon's Citrate Slant, Motility Indole Urease (MIU), Lysine Iron agar (LIA), Urea broth, Indole production, Methyl Red (MR), Voges Proskauer (VP), Nutrient Nitrate Broth (NB), coagulase test with rabbit plasma, DNase test, haemolytic test, Oxidase, and Catalase tests. Identification of isolates obtained in pure culture was based on Gram staining, biochemical characteristics and growth pattern on selective and differential media and; according to the procedures recommended in the Bergey's Manual of Determinative Bacteriology (Holt, 1984; Ewing, 1986).

2.4. Antibiotic susceptibility testing

Twenty two strains were tested for antibiotic resistance by the standard agar disc diffusion technique (Bauer et al., 1966) on Mueller Hinton agar using commercial discs (Oxoid, UK). The antibiotic discs used in this study were: amoxicillin (10 µg), ampicillin (25 µg), bacitracin (10 µg), cefaclor (30 µg), ciprofloxacin (5 µg), neomycin (30 µg), penicillin-G (10 units), streptomycin (10 µg), tetracycline (30 µg), vancomycin (30 µg). A control strain of E. coli ATCC 25922 was included in each plate. Antimicrobial breakpoints and interpretation were taken from the CLSI standards (Clinical and Laboratory Standards Institute, CLSI 2006, formerly NCCLS).

RESULTS

All the *Staphylococcus aureus* isolates showed positive reaction in methyl red, voges-proskauer and catalase test and negative reaction in indole, H_2S production, citrate utilization, motility, urease and oxidase test which are typical biochemical characteristics of *Staphylococcus aureus.*

Table 1: Sensitive (S) Resistance (R) of Staphylococcus aureus isolated from hospital Patients to each of the following antibiotics

Sample ID	P	Amp	S	Tet	Aml	N	Cf	Va	Cec	B
P1	R	R	R	R	R	R	S	S	S	S
P2	R	R	R	R	R	R	S	S	R	S
P3	S	S	R	R	S	S	S	S	S	S
P4	R	R	R	S	R	R	R	S	S	S
P5	S	S	R	S	S	R	S	S	S	S
P6	R	R	R	R	R	R	S	S	S	S
P7	R	R	R	R	R	R	S	S	R	R
P8	S	S	R	S	S	S	S	S	S	S
P9	R	R	R	R	R	R	S	S	S	S
P10	R	R	R	R	R	R	S	S	S	S

P=Penicillin, Amp=Ampicillin, S=Streptomycin, Tet=Tetacycline, Aml= Amoxicillin, N=Neomycin, Cf=Ciprofloxacin, Va= Vancomycin, Cec=Cefaclor, B=Bacitracin

Table 2: Sensitive (S) Resistance (R) of Staphylococcus aureus isolated from teachers to each of the following antibiotics

Sample ID	P	Amp	S	Tet	Aml	N	Cf	Va	Cec	B
T1	R	R	R	R	R	S	S	S	S	S
T2	R	R	R	R	R	R	S	S	R	S
T3	R	S	R	S	S	S	S	S	S	S
T4	S	S	R	S	S	R	S	S	S	S
T5	R	R	R	S	R	R	S	S	S	S
T6	R	R	R	R	R	S	S	S	S	S
T7	S	S	R	R	S	R	S	S	S	S
T8	R	R	R	S	R	R	S	S	S	S
T9	R	R	R	R	R	S	S	S	S	S
T10	R	R	R	R	S	R	S	S	R	S

P=Penicillin, Amp=Ampicillin, S=Streptomycin, Tet=Tetacycline, Aml= Amoxicillin, N=Neomycin, Cf=Ciprofloxacin, Va= Vancomycin, Cec=Cefaclor, B=Bacitracin

Table 3: Sensitive (S) Resistance (R) of Staphylococcus aureus isolated from students to each of the following antibiotics

Sample ID	P	Amp	S	Tet	Aml	N	Cf	Va	Cec	B
S1	R	S	R	R	S	R	S	S	S	S
S2	R	S	R	R	S	R	S	S	S	S
S3	S	S	R	R	S	S	S	S	S	S
S4	R	R	R	S	R	R	S	S	R	S
S5	S	S	R	S	S	R	S	S	S	S
S6	R	R	R	R	R	S	S	S	R	S
S7	R	R	R	R	R	R	S	S	S	S
S8	S	S	R	R	S	S	S	S	S	S
S9	R	R	R	S	R	R	S	S	S	S
S10	S	S	R	R	S	R	S	S	S	S

P=Penicillin, Amp=Ampicillin, S=Streptomycin, Tet=Tetacycline, Aml= Amoxicillin, N=Neomycin, Cf=Ciprofloxacin, Va= Vancomycin, Cec=Cefaclor, B=Bacitracin

Table 4: Sensitive (S) Resistance (R) of Staphylococcus aureus isolated from nurses to each of the following antibiotics

Sample ID	P	Amp	S	Tet	Aml	N	Cf	Va	Cec	B
N1	R	R	R	R	R	S	S	S	S	S
N2	R	R	R	R	R	R	S	S	S	S
N3	S	S	R	R	S	R	S	S	S	S
N4	R	R	R	R	R	R	R	S	R	R
N5	S	S	R	S	S	R	S	S	S	S
N6	R	R	R	R	R	R	S	S	S	S
N7	R	R	R	R	R	R	S	S	R	S
N8	R	R	R	R	R	S	R	S	S	S
N9	R	R	R	R	R	R	S	S	R	S
N10	R	S	R	R	R	R	S	S	S	S

P=Penicillin, Amp=Ampicillin, S=Streptomycin, Tet=Tetacycline, Aml= Amoxicillin, N=Neomycin, Cf=Ciprofloxacin, Va= Vancomycin, Cec=Cefaclor, B=Bacitracin

Table 5: Percentage resistance of the *S. aureus* samples to each of the 10 antibiotics

Groups	P	Amp	S	Tet	Aml	N	Cf	Va	Cec	B
Patients	70 (7/10)	70 (7/10)	100 (10/10)	70 (7/10)	70 (7/10)	80 (8/10)	10 (1/10)	0 (0/10)	20 (2/10)	10 (1/10)
Nurses	80 (8/10)	70 (7/10)	100 (10/10)	90 (9/10)	80 (8/10)	80 (8/10)	20 (2/10)	0 (0/10)	30 (3/10)	10 (1/10)
Teachers	80 (8/10)	70 (7/10)	100 (10/10)	60 (6/10)	60 (6/10)	60 (6/10)	0 (0/10)	0 (0/10)	20 (2/10)	0 (0/10)
Students	60 (6/10)	40 (7/10)	100 (10/10)	70 (7/10)	40 (4/10)	70 (7/10)	0 (0/10)	0 (0/10)	20 (2/10)	0 (0/10)

P=Penicillin, Amp=Ampicillin, S=Streptomycin, Tet=Tetracycline, Aml=Amoxicillin, N=Neomycin, Cf=Ciprofloxacin, Va=Vancomycin, Cec=Cefaclor, B =Bacitracin

It was seen that 0% (vancomycin) to 100% (streptomycin) of the *S. aureus* strains are resistant to the antibiotics used against them. Vancomycin is the best drug for the treatment of *S. aureus* infection. Nurses and Patient samples exhibited a higher resistance rate than normal microflora isolates from Teachers and Students, and there is a great chance of nosocomial infection from nurses.

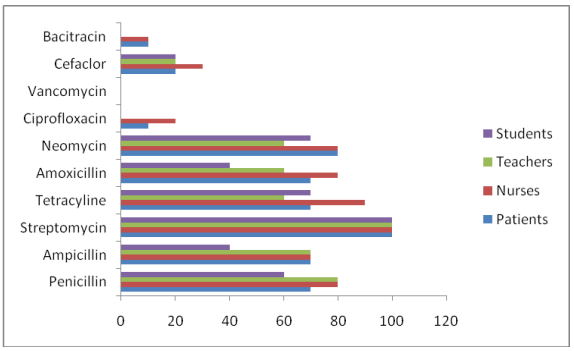

Figure 1: Percentage resistance of the *S. aureus* samples to each of the 10 antibiotics.

Table 6: Percentage of multi-drug resistance of the *S. aureus* samples isolated from various professional groups

	9 drugs	8 drugs	7 drugs	6 drugs	5 drugs
Patients	0	10	20	40	0
Nurses	10	0	20	30	20
Teachers	0	0	10	10	40
Students	0	0	0	30	10

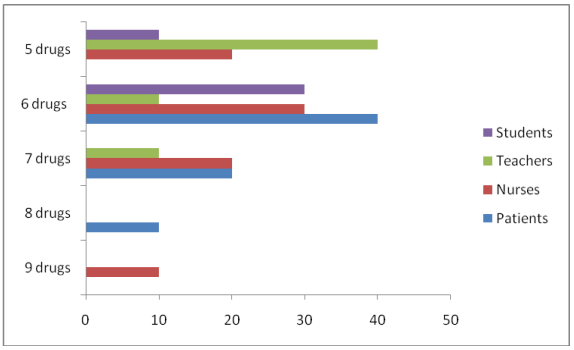

Figure 2: Percentage of multi-drug resistance of the *S. aureus* samples isolated from various professional groups.

DISCUSSION

Staphylococcus aureus is a common cause of skin infection and the pathogen is also capable of living a benign lifestyle in the nasal passage and skin (Wiese-Posselt *et al.*, 2007; El-Gilany and Hanan, 2009). The organism also has great ability to degrade skin lipid barrier and spread within skin loci (Laube and Farrell, 2002; Hoegr, 2004). The frequency of the pathological distribution of *S. aureus* obtained in this study reflects a typical prevalence pattern of the organism in skin.

It was seen that 0% (vancomycin) to 100% (streptomycin) of the *S. aureus* strains are resistant to the antibiotics used against them. Vancomycin is the best drug for the treatment of *S. aureus* infection. Nurses and Patient samples exhibited a higher resistance rate than normal microflora isolates from Teachers and Students, and there is a great chance of nosocomial infection from nurses.

One of the nurse samples was resistant to at least 9 antibiotics, and one of the patient samples was resistant to at least 8 antibiotics. Over 62.5% of the strains are resistant to 5 or more of the antibiotics tested. 100% of the isolates tested were susceptible to vancomycin, where 95.0%, 92.5% and 73.5% of the isolates tested were susceptible to bacitracin, ciprofloxacin and cefaclor respectively.

Prevention of *S. aureus* infections has to date been limited to the application of infection control measures. In some countries, such as the Netherlands and Denmark, where strict isolation policies have prevailed, these precautions have been effective in preventing dissemination of MRSA, while in the USA and England, the success of infection control procedures has been limited. The potential of strict infection control programs to curb MRSA transmission suggests that, given recent trends, stricter infection control guidelines are warranted (Verhoef *et al.*, 1999). Newer, more rapid diagnostic methods that can detect the presence of *S. aureus* or other pathogens and allow for rapid identification and isolation of colonized patients should enhance the efficacy of these programs.

Because nasal carriage of *S. aureus* raises the risk of subsequent infection, efforts have been directed to the elimination of carriage using topical antimicrobials (Yu *et al.*, 1986; Kluytmans *et al.*, 1997; Perl *et al.*, 2002). These approaches have been variably successful. More recently, the potential use of novel agents for this purpose, such as endopeptidase, lysostaphin, or phage lytic enzymes has also been considered (Peacock *et al.*, 2001; Fischtti, 2001; Climo, 1998).

The difficult therapeutic problem of multidrug-resistant *S. aureus* is just one example of the diminishing efficacy of antimicrobial agents for the treatment of bacterial infections. This trend is particularly alarming for *S. aureus* because of the severity and diversity of disease caused by this uniquely versatile pathogen. While effective anti-staphylococcal agents still exist, their shelf-life is likely to be increasingly limited. Novel approaches to therapy and prevention will become more and more important, especially with the diminishing availability of new "wonder drugs."

Since *S. aureus* is easily spread by contaminated hands, strict hygiene practices are needed such as hand washing with soap and warm water as well as good housekeeping. Cover all open wounds with a waterproof occlusive dressing until healed. The use of alcohol-based hand rub solutions in 'clean' situations when hands are visibly clean, particularly when water is not

immediately available, may be useful when travelling or at a picnic, for example. These solutions are not necessary in the home or work situation. There are some situations when alcohol-based hand rub solutions should never be used – for example, instead of washing after going to the toilet. Hands should be washed with soap and warm water and dried.

Finally, several *S. aureus* vaccine candidates are under investigation. A capsular polysaccharide protein conjugate vaccine underwent a clinical trial with hemodialysis patients, with encouraging but inconclusive results (Shinefield *et al.,* 2002). Other candidate vaccines directed at *S. aureus* virulence determinants such as the surface adhesins or enterotoxins are in varying stages of development (Michie, 2002).

Therefore, there is a need to institute strategies for antibiotic resistance surveillance to form the basis for developing and implementing measures that can reduce the burden of infections in our setting.

Relatively little data exists about antibiotic resistance of skin *Staphylococcus aureus* in Bangladesh. Emergence of unusual resistance and similar information is invaluable to clinicians in the case of pathogenic strains; in addition, any resistance thought to have arisen from other sources (such as antibiotic overuse in animal feeds) is of concern from public health aspects. Awareness towards antibiotic regulation should be raised in that case. Also, resistance patterns in hospital-nurse strains will indicate the severity of nosocomial infections that are likely to occur.

CONCLUSION

In conclusion, this study offers primary evidence of prevalence of *Staphylococcus aureus* in skin. Noticeably, *Staphylococcus aureus* remains an agent of recurrent skin infections and the treatment of the infection requires careful evaluation of the commonly available antibiotics especially the beta-lactams. Lack of information on the biodata of the patients, previous antibiotic usage, underlying cause of the infection and genetic profiling studies present limitations to this study and form the basis for further work.

REFERENCES

Bauer AW, Kirby WMM, Snerris JM, Turck M. Antibiotic Susceptibility Testing By a Standardized Single Disc Method. Am J Clin Path 1966;45:493-496.

Boyce JM. Epidemiology and Prevention of Nosocomial Infections. The Staphylococci in Human Disease, Ed. Crossley KB. Churchill-Livingstone, NY. 1997;pp:309-329.

Cabello FC. Heavy Use of Prophylactic Antibiotics in Aquaculture: A Growing Problem for Human and Animal Health and the Environment. Environ Microbiol 2006;8(7):1137-1144.

Climo MW, Patron RL, Goldstein BP, Archer GL. Lysostaphin treatment of experimental methicillin-resistant Staphylococcus aureus aortic valve endocarditis. Antimicrob Agents Chemother 1998;42:1355-1360.

Coplan A, Bodur H, Cevik MA. Evaluation of Antibiotic Use in a Hospital with an Antibiotic Restriction Policy. Int J Antimic Agents 2003;21(4):308-312.

Cosgrove SE, Sakoulas G, Prencevich EN. Comparison of Mortality Associated With Methicillin-Resistant and Methicillin-Sensitive Staphylococcus aureus Bacteriemia: A Meta-Analysis. Clin Infec Dis 2003;36:53-59.

El-Gilany A, Hanan F. Risk Factors of Recurrent Furunculosis. Dermatol Online J 2009;15(1):16

Ewing WH. Edwards and Ewing's identification of Enterobacteriaceae. Elsevier Science Publishing Co., Inc., New York, N.Y. 1986;pp:169-181.

Fischetti VA. Phage antibacterials make a comeback. Nat Biotechnol 2001;19:734-735.

Foster TJ. The Staphylococcus aureus "Superbug". J Clin Invest 2004;114(12):1693-1696.

Hoegr H. Antimicrobial Susceptibility of Skin-colonizing *Staphylococcus aureus* Strains in Children with Atopic Dermatitis. Pediatr Allergy Immunol 2004;15(5):474-7.

Holt JG. Bergey's Manual of Systematic Bacteriology. Williams and Wilkins, Baltimore 1984.

Kluytmans J, van Belkum A, Verbrugh H. Nasal carriage of *Staphylococcus aureus*: epidemiology, underlying mechanisms, and associated risks. Clin Microbiol Rev 1997;10:505-520.

Kluytmans JA, Mouton JW. Nasal Carriage of S. aureus As a Major Risk Factor for Wound Infections After Cardiac Surgery. J Infect Dis 1995;171:216-219.

Laube S, Farrell M. Bacterial Skin Infection in the Elderly: Diagnosis and Treatment. Drugs and Aging 2002;19(5):331-42

Le Loir F, Baron F, Gautier M. *S. aureus* and Food Poisoning. Genet Mol Res 2003;2(1):63-76.

Lowy FD. Staphylococcus aureus Infection. N Eng J Med 1998;339:520-532.

Michie CA. Staphylococcal vaccines. Trends Immunol 2002;23:461-463.

Neu HC. The Crisis in Antibiotic Resistance. Science 1991;257(5073):1064-1073.

Olufemi OL, Olufemi AE, Andrew AO, Omokehinde AH. Antibiotic Susceptibility Pattern and Beta-lactamase Production in Isolates of *Staphylococcus aureus* from Recurrent Furunculosis in Southwestern, Nigeria. Sierra Leone. J Biomed Res 2011;3(3):123-127.

Peacock SJ, de Silva I, Lowy FD. What determines nasal carriage of *Staphylococcus aureus*? Trends Microbiol 2001;9:605-610.

Perl TM, Cullen JJ, Wenzel RP, Zimmerman MB, Pfaller MA, Sheppard D, Twombley J, French PP, Herwaldt LA. Intranasal mupirocin to prevent postoperative *Staphylococcus aureus* infections. N Engl J Med 2002;346:1871-1877.

Shinefield H, Black S, Fattom A, Horwith G, Rasgon S, Ordonez J, Yeoh H, Law D, Robbins JB, Schneerson R, Muenz L, Fuller S, Johnson J, Fireman B, Alcorn H, Naso R. Use of a *Staphylococcus aureus* conjugate vaccine in patients receiving hemodialysis. N Engl J Med 2002;346:491-496.

Shopsin B, Kreiswirth BN. Molecular Epidemiology of Methicillin-Resistant *Staphylococcus aureus.* Emerg Infect Dis 2001;7:323-326.

Verhoef J, Beaujean D, Blok H, Baars A, Meyler A, van der Werken C, Weersink A. A Dutch approach to methicillin-resistant *Staphylococcus aureus.* Eur J Clin Microbiol Infect Dis 1999;18:461-466.

Von Eiff C, Becker K. Nasal Carriage as a Source of *Staphylococcus aureus* Bacteremia. N Eng J Med 2001;344:11-16.

Wiese-Posselt M, Heuck D, Draeger A, Mielke M, Witte W, Ammon A and Hamouda O. Successful Termination of a Furunculosis Outbreak Due to LukS-LukF-Positive, Methicillin-Susceptible *Staphylococcus aureus* in a German Village by Stringent Decolonization, 2002-2005. Clin Infect Dis 2007;44:e88-e95

Yu VL, Goetz A, Wagener M, Smith PB, Rihs JD, Hanchett J, Zuravleff JJ. *Staphylococcus aureus* nasal carriage and infection in patients on hemodialysis. Efficacy of antibiotic prophylaxis. N Engl J Med 1986;315:91-96.