Urinary Tract Infections in Hospital Pediatrics: Many Previous Antibiotherapy And Antibiotics Resistance

Khaldi A.[1], Tifrit A[1] ,Chama Z[1], Abbouni B[1], Rebahi H, Zinai R ,Tir touil A.'[2],

Abstract: We studied pediatric antibiotic resistance and evaluated the impact of antibiotic exposure in the previous 10 months, with very little available Algerian data for this population. We conducted a prospective, multicenter study involving children who were admitted or admitted to the sidi bel abbes hospital in western Algeria. Pre-exposure to antibiotics was documented from their medical records. Of 75 patients (40 girls), 2 to 12 years, were included in 10 months. Ninety-five percent had pyelonephritis associated with 25% uropathy. Escherichia coli was predominant (78%). In Gram-positive bacteria, Staphylococcus aureus was most frequently isolated with 23%. Among the Gram-negative bacteria, Klebsiella sp, Salmonella sp were the most frequently encountered organisms with a prevalence of 20%, 15% and 12%, respectively. However, Shigella sp and Providencia sp were found at 10% and 08% in different samples. In other Gram-negative bacteria, Pseudomonas aeruginosa was isolated at 12%. Followed by Proteus spp. And Enterococcus spp. The antibiotic resistance rate of E. coli was high and close to that reported for adults with complications: 60% amoxicillin, 35% amoxicillin-clavulanate, 5% cefotaxim, 26% sulfametoxazole trimethoprim, 9% nalidixic acid, ciprofloxacin 7 %, Gentamycin 1%, nitrofurantoin and fosfomycin 0%. Exposure to antibiotics in the past 12 months was most frequent with 62 children (56%) with β-lactams (89%) for respiratory tract infection (56%). The bacterial species and the level of resistance to antibiotics in children are similar to those reported in adults.

Our results indicate that all strains isolated and identified in various sites were multiresistant. Antibiotics 50% of strains isolated from Staphylococcus aureus are presumed to be MRSA. In the Gram Negative and Enterobacteriaceae 79.46% isolated strains producing ESBL. Exposure to antibiotics in the past 12 months increases the risk of resistance but other factors are involved (previous antibiotic therapies and fecal-oral or mother-to-child transmission).

Keywords : Bacterial resistance, Pediatric Urinary , tract infection

I. Materials And Methods

A. *Samples collection*

This study was conducted between March and December 2016 from thz hospital of sidi bel abbes western north of Algeria. A total of 75 patients (40 girls), 2 to 12 years of age, 75% presented with pyelonephritis, associated to uropathy for 25%.

The urines were transported to the laboratory and stored at –20°C until further analysis.

B. *Bacterial isolation and identification*

All samples from urinary infections, were prepared by diluting scraped cell mass in 0.85% NaCl sterile solution and then analyzed for aerobic bacterial content by cultures on a series of non-selective and selective media (Blood agar, Chapman medium, Hektoen medium, Nutritive agar medium. Plates were incubated at 37°C for 24 and 48 h, grown colonies and pure cultures were identified by recommendation of Bergy's [6] using standard morphological, Gram coloration and biochemical methods with commercials kits (API Staph, API 20 E Biomerieux, Marcy l'Etoile, France). The tests were conducted twice for each sample and the mean of Colony Forming Unit (CFU) count was determined. The microbial strains were maintained on agar slant at +4°C until analysis.

C. *Inoculums' preparation*

Nutriment broth [7] was used for growing strains and diluting suspensions. Bacterial strains were grown to exponential phase in nutriment broth at 37°C for 18 h and adjusted to a final density of 2x108 CFU by diluting fresh cultures and comparison to Mac Farland standards (OD650=0.7) [8].

D. *Antibiotic susceptibility testing*

Resistance towards antibiotics was assessed for each strain with the disc diffusion method [9] and bacterial growth on Muller Hilton Agar plates. The antibiotic tested for Enterobacteriaceae were Ampicillin (10 µg), Gentamicin (10 µg), Aztreonam (30 µg), Colistin (10 µg), Tetracyclin (30 µg), Chloramphenicol (30 µg) Amoxicilline(), cefotaxin Acide nalidixique () ciprofloxacine amoxicilline – clavulanate() trimethoprime-sulfametoxazole() for Staphylococaceae, were Oxacillin (10 µg), Erythromycin (15 µg), Spriramycin(10 µg), Chloramphenicol (30 µg), Tetracyclin (30 µg),

E. *Staphylococcus aureus resistant Methicillin (MRSA) search*

Dip a sterile swab into the bacterial suspension standardized at 106 CFU / ml. The swab is rubbed on the agar surface of the screen (Muller Hinton). Submit a oxacillin 10 µg the center of the box hard and incubated for 24 h at 37°C isolated in the presence of the inhibition zone around the disk colonies resistant MRSA and heterogeneous [10]

[1]Laboratoire de Microbiologie Moléculaire,Protéomics et Santéuniversité de sidi bel abbes.- Algérie

[2]Laboratoire de Recherche sur le système biologique et géomatique Faculté SNV, université Mustapha Stambouli Mascara-Algérie

***Correspondance:** amkhaldia@hotmail.com,00213 552982143

Photo 1: *Staphylococcus aureus* resistant for Methicillin

β- lactamases search in gram negative bacteria

Phenotypic demonstration of the presence of a β-lactamase extended spectrum in Enterobacteriaceae is to highlight an image of a disk synergy between third-generation cephalosporin and clavulanic acid. Apply on Mueller Hinton agar [10] previously
seeded with the test strain, a disc of cephalosporin 3rd generation: (aztreonam (ATM) or ceftazidime (CAZ), or cefixime (CFM) or cefotaxime (CTX), after incubation for 18 h at 37°C .

Search for a penicillinase by the iodometric method

This method was described by Perret in 1954. The principle is based on the destruction of the complex by penicillic acid resulting from the attack of penicillin by penicillinase.
Preparation of reagents
•Substrate Dissolve penicillin G in 0.1 M phosphate buffer at pH 6 so as to obtain a concentration of 6 mg / ml.
•Starch solution
Add 1 g of starch to 100 ml of distilled water and heat in a bain-marie until completely dissolved.
•iodo-iodide reagent
Dissolve 2.03 g of iodine and 53.2 g of potassium iodide in a small volume of distilled water and then make up to 100 ml. Store in a tinted glass bottle. Penicillin and starch solutions should be prepared on the day of testing. Distribute sterile haemolysis tubes to a rack and dispense 0.1 ml of penicillin G solution into each of them; Prepare a very dense suspension of the strain to be tested and add it to the substrate in the cup, then leave at laboratory temperature for 30 minutes; Add 2 drops of starch solution and mix; Add 1 drop of lugol; A very dark blue or purple color develops immediately following the reaction between lugol and starch. Stir the mixture for one minute at laboratory temperature; A discoloration occurring less than 10 min afterwards translates the production of penicillinase (Boussoualim., 2011).

The detection of carbapenemase (Hodge Test)

The modified version of the Hodge test, a phenotypic test initially developed to detect penicillinases, is widely used for the detection of carbapenemases (Giske CG et al., 2011).
NOTE
The strain of Escherichia coli ATCC 25922 served as the reference strain for this test.
This test consists in seeding inoculum standardized at 106 CFU / ml of the sensitive reference strain Escherichia coli ATCC 25922 on a Muller Hinton agar in a confluent culture (with a swab);
Then an imipenem disk loaded at 10 µg is deposited in the center of the box;
Each strain tested is seeded radially from the disc to the edge of the Petri dish (Giske CG et al., 2011).

F. Statistical Analysis

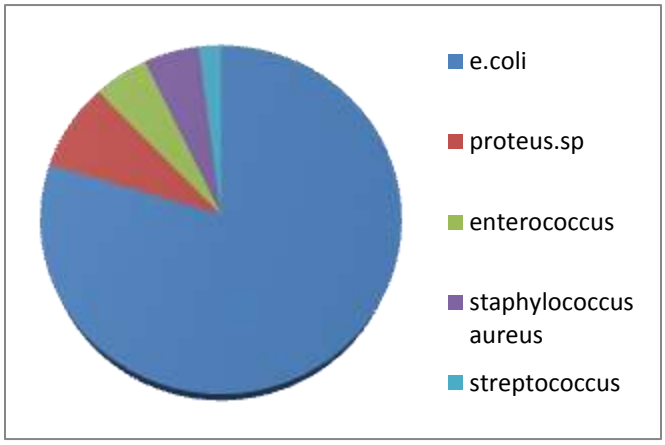
All experiments were made in duplicate. The tests were conducted twice for each sample and the mean of Colony Forming Unit (CFU) count was determined. The results are presented as the mean ± SD. The frequency of strains isolated and antibiotic sensibility test were calculated as percentage.

II. Results

A. Pathogens bacteria isolated from different samples

In Total 80 strains were isolated frome urinary infections According to the results, a large dominance of Gram-negative represented by 80% were founded against 20% of Gram-positive bacteria (Figure 1).

We notice that in the Gram negative bacteria, *E.coli* was the most frequently isolated with 78% (Figure 2). Among the Gram positive bacteria, *staphylococcus aureus* 5% However *streptococcus* 2 % and 05% in different samples (Figure 2). In other Gram negative bacteria, *Pseudomonas aeruginosa* was isolated (Figure 2). This results was the same as reported in a study of Garraffo A and al. [11].

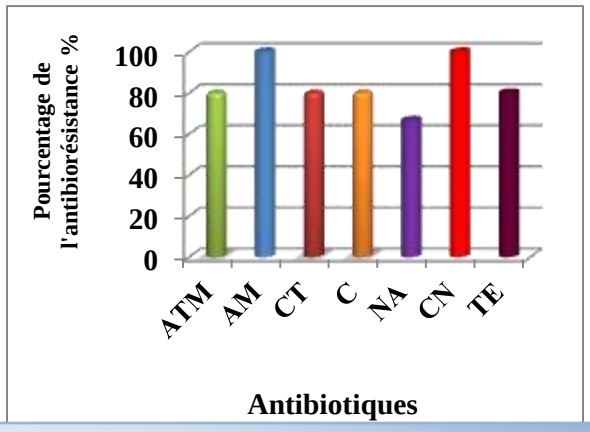


Prevalence of strains in the urinary infections

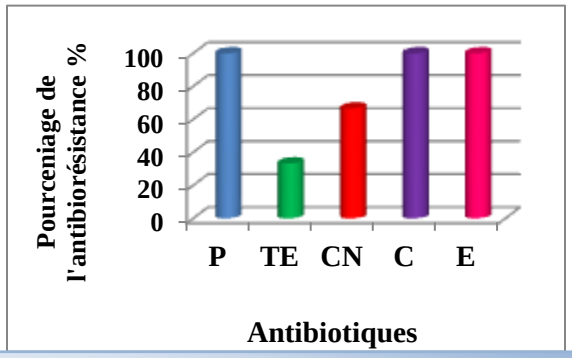
Antibiotic sensitivity profile of isolated pathogens strains

The profile of antibiotic resistance in bacteria isolated from various urinary infection settings shows that most Gram negative strains have a total resistance to Ampicillin and Gentamicin and 80% to the aztreonam, while they showed varying resistance to other antibiotics tested (Figure

4a). However, all gram-positive strains have a total resistance to penicillin and chloramphenicol and erythromycin variable resistors other antibiotics tested (Figure 4b). Our results related to the susceptibility of isolated bacteria to antibiotics, show that E. coli isolated from the clinical infection showing a trend of increasing resistance to the majority of antibiotics, which is consistent with the results of studies in other countries such as France, the United States and Tunisia [8,9,12,13]. Klebsiella is naturally resistant to ampicillin, it has acquired other types of resistors which may act in a way giving simultaneous multi strains very high strength. Comparing the rate of antibiotic resistance in Klebsiella sp to antibiotics tested is common in 1993 and 2010, shows that this bacteria has developed more resistance during this period of time especially for ceftazidime, whose rate resistance has increased from 00-39%, and for the fluoroquinolone from 10 to 27%) which showed a highly correlation with our study [11]. The evolution of bacterial resistance to antibiotics over time
is illustrated by comparing the profiles of resistances implicated the nosocomial infections. In this study we have shown that in a time interval, the increase of the resistance against a various families of antibiotics is multiplied which were confirmed by Rhazi Filali [13]. The acquisition by bacteria, means to fight against the lethal effect of antibiotics or genotypic changes that result, progress in the same direction as this resistance making the bacteria more virulent [11].

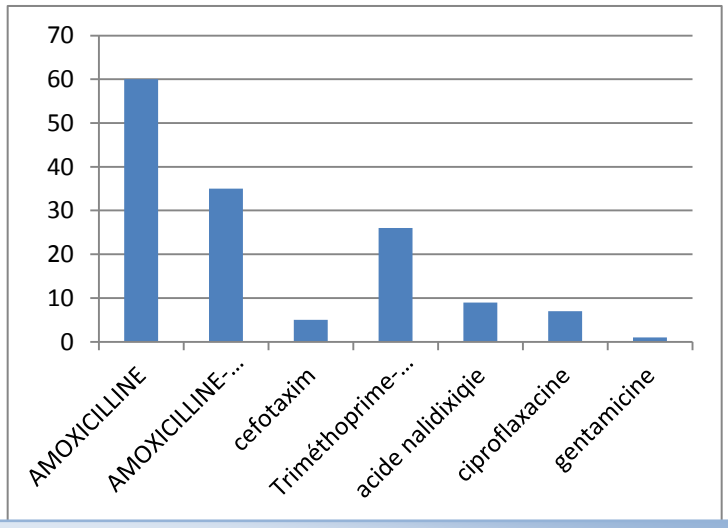


Percentage of antimicrobial resistance of Gram-negative bacilli isolated from urinary tract infections



Percentage of antimicrobial resistance of Gram-positive cocci isolated from urinary tract infections

The profile of antibiotic resistance in E . coli isolated from urinary infections settings shows that most Gram-negative strains have a total resistance to Amoxicilline 60% and amoxicilline-clavulanate 35% triméthoprine-sulfametaxozole 26% acid nalidixique 9% ciprofloxacine 7% and Gentamicin 1% , while they showed varying resistance to other antibiotics tested (Figure 4a). However, all gram-positive strains have a total resistance to penicillin and chloramphenicol and erythromycin variable resistors other antibiotics tested (Figure 4b). Our results related to the susceptibility of isolated bacteria to antibiotics, show *that E. coli* showing a trend of increasing resistance to the majority of antibiotics, which is consistent with the results of studies in other countries such as France, the United States and Tunisia [8,9,12,13].



Percentage of antimicrobial resistance of E.coli

Detection of MRSA

Among strains of *Staphylococcus aureus* isolated from different specimen 50% are presumed MRSA (Photo 03). MRSA is resistant to methicillin by acquiring a gene producing a modified penicillin binding protein (PBP2a). This protein is encoded by the mecA gene locates on a mobile genetic element. It acts as transpeptidase linking peptidogly can essential to the membrane structure of the bacterial cell [14]. The PBP2a are different from regular PLP their very low affinity for antibiotics with a β-lactam ring. For this reason, penicillins, cephalosporins and other β-lactam antibiotics are not effective against MRSA, and cross-resistance occurs with clindamycin, the Carbapenems, macrolides and tetracyclines. Vancomycin is a possible alternative first-line [14]. Since the implementation of a Federal Program Nosocomial Infection Surveillance [15] in 1995, the incidence of MRSA in hospitals has increased by 20, from 0.46 per 1,000 admissions in 1995 to 9.5 per 1000 admissions in 2009 [15].

Detection of beta-lactamase in Gram negative bacteria

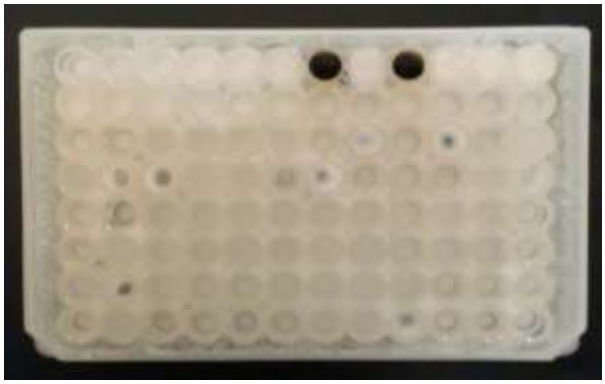
The result of a positive test is the inhibition zone around the discs cefixime or aztreonam in the presence of amoxicillin and clavulanic acid [16]. All strains isolated from different specimen and resistances to antibiotics were tested for the production of beta-lactamase using the spread spectrum method previously described double halo. The results indicates that the most isolated strains (79.46%) producing ESBL, which are mainly *Escherichia coli*, During antibiotic therapy, the acquisition of resistance determinant and the selection of resistant subpopulations in patients initially infected with a susceptible strain, presents a major problem [17]. The present study has shown that all strains are multiresistants. Previous studies have shown that ESBL-mediating plasmids may carry more than one beta-lactamase gene and that they may be responsible for high-level beta-lactamase resistance phenotypes [18]. The major risk in the beta-lactamase genes

The direct and indirect costs associated with antibiotic resistance; promote national coordination and development of action plans to respect the measures of hygiene, combat antibiotic resistance; promote the prudent use of antibiotics and the systematic implementation of infection control measures for the prevention and treatment for reduce morbidity, mortality.

The use of antibiotics in human health and animal, including the impact on the food chain; review the teaching of prudent use of antibiotics in the faculties of medical sciences, veterinary and life, and implement effective policies.

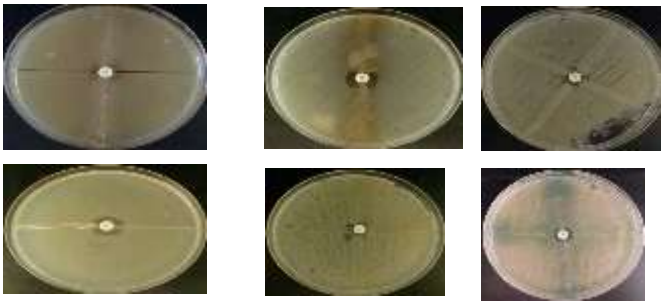
Detection of Penicillinase

On the E. coli strains tested by the iodometric method, penicillinase-like β-lactamase was found to be 98.26%, of course after detecting discoloration which took place at least 10 min, But the rest of the strains show no visible ability to degrade penicillin G, as the initial color remains intact.



Carbapenemase Detection

The deformation of the diameter at the intersection between a streak and the culture of Escherichia coli ATCC 25922 indicates the presence of hydrolysis of the carbapenes by the strain tested (Lee K, Chong Y., 2001).



Our study indicates that most strains isolated from all three sites are producing

Carbapenemases (81.81%) which is mainly produced by bacterial species that are (Escherichia coli, Klebsiella pneumoniae, Shigella sp, Enterobacter spp, Serratia marcescens and Citrobacter freundii) To 100% while the other species are variable.

Beta-lactamases with carbapenemase activity are the most potent mechanisms of resistance to carbapenems. These Carbapenemases are increasingly identified in Enterobacteriaceae worldwide.

However, their presence is often coupled with the presence of extended spectrum β-lactamase (ESBL), which leads to a multiresistance of secretory strains (Boutet-dubois A et al., 2012).

The mechanism that can cause resistance of Enterobacteriaceae to Carbapenems: production of Carbapenemases belonging to different classes of β-lactamases (Ambler classification): class A (KPC), class B (metalloenzymes VIM, IMP, NDM) D (OXA-48, OXA-23, OXA-181 and variants thereof) (CA-SFM., 2012).

In 2005, in the United Kingdom, the Health Protection Agency issued a first alert to sensitize laboratories to the emergence of carbapenemase production by enterobacteria by various mechanisms: Metallo-β-lactamases (MBL), KPC and OXA enzymes -48. Since then, the number of isolates of Enterobacteriaceae producing carbapenemases has been increasing. Isolates producing emerging metallo-β-lactamase. The bacteria involved are mainly Klebsiella pneumoniae and Escherichia coli.

III. CONCLUSION

Our study over a short period raised significant data on the alarming development of resistance and the emergence of multidrug-resistant bacteria to antibiotics. Indeed all of the isolated bacterial strains (80) has a significant increasing trend with multidrug resistance over time as a consequence of improper use of antibiotics.

The testing for beta-lactamase reveal the presence of ESBL (which reached 100% in MRSA) of penicillinase and carbapenemase in most organisms isolated. However, resistance by producing penicillinase is the type of resistance the most common.

In general, and to reduce the selection pressure of antibiotics that encourages the emergence, proliferation and spread of ESBL Enterobacteriaceae, it should reduce the volume of antibiotics used in humans by intensifying actions in part of the antibiotic level. We must, in particular, introduce, next to the concept of "good use", the concept of "any use". A strong proposal to advance in this area is to define, document and make the situations in which it is recommended not to prescribe antibiotics (Rabaud Ch., 2010).

IV. REFERENCES

[1] Chavez and al., 2001 Chavez J, Ladona MC, Segura C, Coira A, Reig R, V. 2001 Ampurdanés SHV- 1 beta-lactamase is chromosomally encoded Mainly a species-specific enzyme in Klebsiella pneumoniae. Antimicrob. Agents Chemother 45: 2856-2861.

[2] Colin, M. (2002) Salmonella spp. Microbiological sheet AFSSA (online) .House-Alfort: French Agency for Sanitary Food Safety. P 6.

[3] BCTC., 2004 Toulouse Centre for Quality control in clinical biology. MSDS: Proteus mirabilis ESBL. Issued October 12, 2004.

[4] BCTC., 2007 Toulouse Centre for Quality control in clinical biology. MSDS: Enterococcus faecalis. Issued June 5, 2007.

[5] BCTC., 2007 Toulouse Centre for Quality control in clinical biology. MSDS: Shigella flexneri. Issued November 12, 2007.

[6] BCTC., 2009 Toulouse Centre for Quality control in clinical biology. MSDS: S. aureus. Issued July 10, 2009.

[7] BCTC., 2011 Toulouse Centre for Quality control in clinical biology. MSDS: Enterobacter cloacae. Issued August 3, 2011.

[8] BCTC., 2011 Toulouse Centre for Quality control in clinical biology. MSDS: Pseudomonas aeruginosa. Issued May 17, 2011.

[9] BCTC., 2012 Toulouse Centre for Quality control in clinical biology. Technical details: Escherichia coli. Issued November 26, 2012.

[10] Cuzon G, Naas T; Nordmann P; 2010 carbapenemase KPC type: What is at stake in clinical microbiology. Original article. , 2010.

[11] R.N. Das, Chandrashekhar j. Ts, Gurung m, n and Shivananda shrestha pg. Frequency and susceptibility profile of pathogens Causing Urinary tract infections at a tertiary care hospital in western nepal, 2006, Singapore med j 47 (4). 281-285.

[12] Jonas D, Spitzmüller B, Daschner FD, Verhoef J, Brisse S. 2004 Discrimination of Klebsiella pneumoniae and Klebsiella oxytoca phylogenetic groups and Klebsiella species --other by use of amplified fragment length polymorphism. J Reseach in Microbiology 155: 17-23.
Kebe, 2001 Issa Kebe. Portage enterococci Resistants a • the • vancomycin • in humans and in animals. Thesis for the degree of Doctor of pharmacie.2001.

[13] John W. KING, 1995 Antibiotic resistance Bug Bytes Vol.2, No. 13 October 4.1995 susceptibility testing of the French Society for Microbiology. Recommendations 2007

[14] Lee K, Chong Y, Shin HB, YA Kim, Yong D, Yum JH, et al. EDTA-disk synergy tests to screen metallo-beta-lactamase-producing strains of Pseudomonas and Acinetobacter species. Clin Microbiol Infect 2001; 7: 88-91.

[15] Lepelletier. D meticillin-resistant Staphylococcus .: aureus: incidence, Risk Factors and interest of systematic screening for colonization in intensive-care unit, 2006. 25 (6): 626-32.

[16] Li. X. Z, Mehrotra. M.Ghimire. S, Adewore. L.2007. Beta-Lactam resistance and beta-lactamases in bacteria of animal origin. Veterinary Microbiology, 121.197 to 214.

[17] MAINARDI JL, Goldstein FW, L. Gutmann, 1996 Mechanism of bacterial resistance to antibiotics. Encycl Med. Chir (Elsevier, Paris), Infectious Diseases, 8.006- N -10.

[18] Mandell GL, Bennett JE, Dolin R. Mandell., 2009, Douglas and Bennett's principles and practice of infectious diseases. Sixth edition, Elservier, Churchill Livingstone Publishers, USA.

[19] Ramata Mariko., 2005 and bacteriological character instead of Streptococcus pneumoniae in invasive bacterial infections in children hospitalized in the pediatric ward of the hospital Gabriel Tour. Thesis for the Degree of Doctor of Pharmacy, State Diploma.

[20] Maryse Archambaud Danielle Clave., 2008 Bacteriological Laboratory Hygiene Hospital. Toulouse

[21] Mastouri M., M. Nour, Mr. Ben Nejma, Bouallegue O., M. Hammami, Khedher M., 2009 Pathology biology. vol 54, issue 1: 33-36.

[22] Matthieu Eveillard, 2007 Policy screening for methicillin-resistant Staphylococcus aureus on admission. Adaptation to the diversification of risk factors portage consequences of this policy for monitoring indicators and transmission. PhD thesis.

[23] MBVB., 2011 Mention "Microbiology-Plant Biology and Biotechnology" Teaching Unit "Introduction to Research in Microbiology." Issue of technical principles.

[24] A. Merlet, 2010 Involvement of Panton Valentine leukocidin in severe Staphylococcus aureus infections in New Caledonia, Audrey MERLET, October 4, 2010