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# Screening and sensitivity of non-lactose fermenting bacteria to antibiotics by Vitek-2 compact system

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## Abstract:-

We are collected 150 clinical sample from different private veterinary clinics in Basrah province . Included 25 isolates from nasal discharge, 45 from feces , 35 from pus/wound infections, 20 from blood and 25 from ear swab .All isolates were subjected to microscopic examination and diagnosed 4 species of Non- Fermenting Gram Negative Bacilli (NFGNB) bacteria based on 47 biochemical tests by Vitek 2 a compact system where the species were 48 strain (32%) of Pseudomonas aeruginosa , 32 strain (21.33%) of Burkholderia cepacia, 31strain (20.66%) of Sphingomonas paucimobilis, 20 strain (13.33%) of Proteus mirabilis and 19 strain (12.66%) of Acinetobacter calcoaceticus . NFGNB results showed antibiotic susceptibility to different antibiotic resistance and susceptibility, where isolates showed Pseudomonas aeruginosa, Burkholderia cepacia and Proteus mirabilis were susceptibility to antibiotic Meropenem (80% -98%), while Acinetobacter spp, Pseudomonas aeruginosa least susceptible to Cefuroxime and Trimethoprim/Sulfamethoxazole (20% – 30%). The bacteria showed Sphingomonas paucimobilis showed resistance to all the antibiotics tested. The current study was carried out with the aim of diagnosing and identifying NFGNB isolated from clinical samples and evaluating their clinical importance and study of antibiotic resistance.

**Key word:-** Non- Fermenting Gram Negative Bacilli (NFGNB), Vetik-2 compact, Identification, Drug resistance.

## Introduction:-

Fifteen percent account of (NFGNB) all bacteria isolates from a clinical Microbiology Laboratory (Gokala and Metgud 2012, Rit etal., 2013, Patal 2013 and Kirthilaxmi and Benachinmardi 2014). Ubiquitous of NFGNB are everywhere in the atmosphere and it in charge for a enormous diversity of infections (Dijkshoom etal., 2007 and Lipuma etal., 2007). and it's chiefly connected with urinary tract infections, ventilator associated pneumonia (VAP), surgical site infections and bacteraemia ,the important health be concerned related pathogens of NFGNB have emerged (Bergogne and Towner 1996). Several automated systems fortified susceptibility of bacteria in clinically important are a viable (Stager and Davis 1992 and Robinson et al ., 1995).

For the facilitate the identification of bacteria gram negative by used the Vitek-2 compact system to detect metabolic changes by fluorescence based methods within 6 hours, this system mechanism of it's action monitors the kinetics of bacterial increase and calculate Minimum Inhibitory Concentration (MIC) by using a unique algorithm (Joyanes et al., 2001 ).

## Materials and Methods:-

One hundred fifty samples collected from different clinical samples from different private Veterinary clinics in Basrah province. Only (NLF) Gram Negative bacteria that grew well in MacConky agar were included. Inoculums preparation – beginning the isolated colonies grown on the media, a bacterial suspension was prepared in 3 mL of sterile saline (aqueous 0.45% - 0.50% NaCl, pH 4.5 to 7.0) in a 12x75 mm clear plastic (polystyrene) test tube, the turbidity of the suspension was used to a McFarland standard of 0.5 with the help of a VITEK-2 Densi Check instrument(Jual et al., 2013 ). The time between the preparation of inoculums and heavy of the certificate was forever less than 30 min.

Identification with the VITEK-2 compact system was perform using a Gram Negative (GN) card according to the Manufacturer's instructions (Joyanes et

al., 2001). The 64 well plastic GN card contains 41 tests including 18 tests for sugar incorporation, 18 tests for sugar fermentation, 2 decarboxylase tests and 3 miscellaneous tests (for urease, tryptophan deaminase and utilization of malonate).

**Antimicrobial susceptibility testing:-**

This test with the VITEK-2 compact system was performed by means of an AST N281 card according to the manufacturer's directions. The VITEK-2 AST N281 vulnerability card is proposed for use with the VITEK-2 systems in clinical laboratories as an in-vitro test to decide the vulnerability of clinically significant aerobic gram negative bacilli to ntimicrobial agent (Simgamsetty et al., 2016 and Ling et al., 2003). Antibiotics tested in AST N281 card incorporated Cefepime, Levofloxacin, Meropenem, icarcillin/Clavulinic acid, Gentamicin, Imipenem, Ceftazidime, Doripenem, Cefoperazone/Sulbactum, Ciprofloxacin, Minocycline, Tigecycline, Amikacin, Trimethoprim/Sulfomethoxazole (Cotrimoxazole), Colistin, Tobramycin, Piperacillin /Tazobactum, Cefuroxime, Ceftriaxone, ephotaxime, the cards were overflowing with an inoculums (ready by transfer 200µL of culture suspension from the 0.5 McFarland culture suspension used for satisfying the identification cards into a clean 3mL sterile saline solution obtain a concluding turbidity of 8x106 cfu/mL) in the satisfying hall, the VITEK-2 System automatically process the antimicrobial susceptibility cards until MIC's are obtained, the VITEK-2 compact system next correct, where essential for MIC's or clinical group in agreement with the interior folder of likely phenotypes for microorganism antimicrobial agent combination (OHara et al.,1997 and Bruno et al.,2011).

## Results:-

A total of 150 strains collected from various clinical specimens were tested in VITEK-2 compact system, out of the 150 strains included 25 strains were isolated from nasal discharge, 45 from feces , 35 from pus/wound infections, 20 from blood, 25 from ear swab figure (1).

All isolates were subjected to microscopical test and diagnosed 4 species of (NFGNB) bacteria based on 47 biochemical tests by Vitek 2 a compact system where the species 48 strain (32%) of P. aeruginosa , 32 strain (21.33%) of B. cepacia , 31strain (20.66%) of Sph.. paucimobilis, 20 strain (13.33%) of Pro. mirabilis and 19 strain (12.66%) of Acin.. calcoaceticus as shows ( Figure 2) . Discrepant results were determined by liability added biochemical tests. P. aeruginosa strains were most vulnerable to Meropenem (80%) and least susceptible to Cefuroxime and Trimethoprim/Sulfamethoxazole (20%). Strains of Acinetobacter spp. were most susceptible to Colistin (90%) and lest susceptible to Cefuroxime and Trimethoprim/Sulfamethoxazole (30%). B. cepacia were establish to be sensitive to Levofloxacin, Meropenem, Minocycline, Trimethoprim/ Sulfamethoxazole, and Ceftriaxone (100%). Prot. mirabilis strain were most vulnerable to Ceftriaaxone , Meropenem and (98%) while, Sphingomonas paucimobilis show resistance to all the antibiotics tested figure (2).

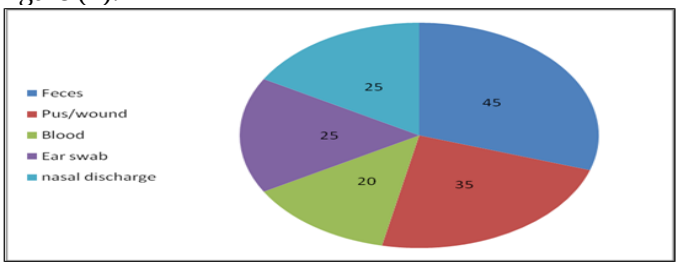


Figure 1 : Diagram showing the allocation of specimens used in the study

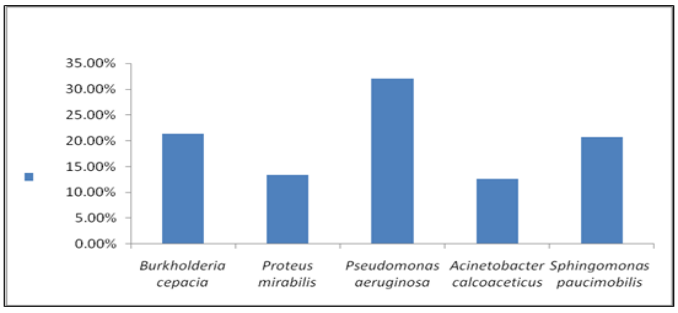


Figure 2: Diagram showing the allocation of bacteria variety isolate in the study.

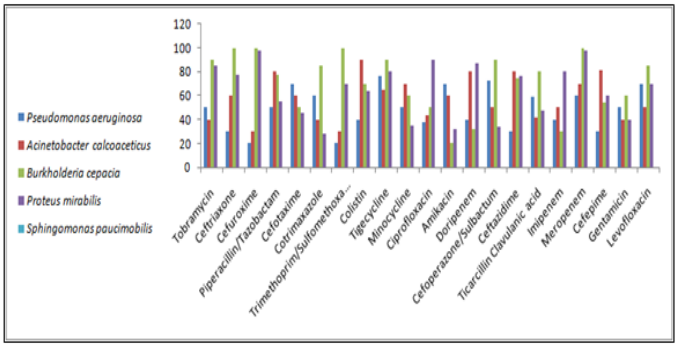


Figure 2: Diagram showing the proportion sensitivities of the (NFGNB) towards a variety of antibiotics

## Discussion:-

The results in our current study showed that 4 species that develop into NFGNB include *P. aeruginosa, B. cepacia, Sph. Paucimobilis* and *Pro. Mirabilis* .The important pathogens NFGNB which were beforehand careful to be contaminants have now emerge(Funke *et al*., 1998). *P. aeruginosa* a*nd Acinetobacter* species are known to be ordinary amongst them( OHara *et al*.,1997). The VITEK-2 compact system includes numerous compensation which may be of clinical attention for routine testing of gram negative rods that isolated from clinical sample like a simple method, fast identification, taxonomically efficient database and a high level of mechanization(Kumari *et al*.,2007). In our studies that isolates *p. aeruginosa* were susceptible to Meropenem, Cefoperazone/Sulbactum, Doripenem, Cefepime, Levofloxacin and Amikacin that alike to the previous studies conduct by(Shashwati *et al*., 2014) *Acinetobacter* species strains show high percentage resistance to Ceftazidime, Ciprofloxacin and Amikacin in an previous(Kumari *et al*.,2007), that were in association to the results present in our study(Bruno *et al*., 2011). Susceptibility pattern may be distorted due to resistant mutant and transfer collection from excessive use of antibiotics and indiscriminate(Gilardi 1971 and Von Gravenitz 1973). Therefor, the high rate of resistance of *P. aeruginosa* to carbapenems, piperacillin-tazobactam and amikacin have been describe( Manoharan *et al*., 2010 and Pawar *et al*., 2008), most isolates in our location which susceptible to primary aminoglycosides, antipseudomonals and the carbapenems. *P. aeruginosa* isolates of in our study retain clinically helpful vulnerability to aminoglycosides (amikacin, gentamicin) and it have an important role to play in the antibiotic action of these organisms in our set up. An additional study, showed a inferior vulnerability of *P. aeruginosa* to amikacin and imipenem compared to our observation ( Jamshidi *et al*., 2009).

These occurrence of multidrug resistant pathogenic organisms in recent study has enlarged particularly, therefore healing proposal have been customized according to the appearance of drug adapted and resistance to epidemiological marker of character infectious process, environmental variation of these the availability and markers of new antibacterial agents( Alverez- Lerma *et al*., 2006). Sensitivity *B. cepacia* to trimethoprim –sulfamethoxazole was in harmony with( Tseng *et al*., 2014), while differ with(Omar *et al*., 2015). Who report sensitivity rates (0%, 43.6%) respectively . Concerning quinolones sensitivity, results indicate the proportion of sensitivity to Ciprofloxacin50% and Levofloxacin 85%, the resistance of *B. cepacia* to many antimicrobial agents and its resistance to β-lactam antibiotics the majority usually results from mixture of constitutively expressed and impermeability or inducible chromosomal β-lactamases or efflux pump, fewer usually, plasmid-mediated β-lactamases of the TEM class are (cephalosporinases) award resistanse to β-lactam antibiotics( Roy et al., 2014). The inherent resistance *B. cepacia* to aminoglycosides which owing to not have of required sites on the lipopolysaccharide, condensed external covering efflux pumps and permeability (Cox and Wilkinson 1991). The results in this study was agreed with Colstein has showed that a higher activity with *Acinetobacter spp*, than Imipenem or cefoperazone/sulbactam, mind in discovery, assessment of efficient antibiotic option, and sensible use of antibiotics by institute antibiotic rule for mixture treatment and exact infection control actions will help us to brawl alongside these multidrug resistant NFGNB( Leepethacharat and Oberdorfer 2007).

## Conclusion:-

The good identification of NFGNB up to the species level which important for correct management of the infection .Care in evaluation, detection of effective antibiotic options, and sensible use of antibiotics by instituting antibiotic rule for mixture therapy and exact infection control events that help us to brawl next to these multidrug resistant NFGB. P.aeruginosa showed good quality sensitivity to ciprofloxacin , amikacin and meropenem,. Burkholderia cepacia showed good sensitivity to Levofloxacin, Meropenem,Minocycline,rimethoprim Sulfamethoxazole, and Ceftriaxone

## Reference:-

Álvarez-Lerma, F.; Alvarez, B.;Luque, P.; Ruiz, F.; Dominguez-Roldan, JM.; Quintana, E. and Sanz-Rodriguez, C. (2006). Empiric broad-spectrum antibiotic therapy of nosocomial pneumonia in the intensive care unit: a prospective observational study. Crit. Care 10(3):78.

Bergogne – Berezin, E.andTowner, KJ. (1996). *Acinetobacer spp* as Nosocomial pathogens: Microbiological, Clinical and Epidemiological features. Clin Microbial Rev.;9(2):148-65.

Bruno, D.; Nishano, MK.; Prior, WN.; Remus, PR. and Do Cormo AA. (2011). Prevalence of Gram negative nonfermenters Patients in proto Alegre – RS. Journal Brasileiro de Patologia e Medina Laboratorial. 47:529-34.

Cox, A. D. and Wilkinson, S. G. (1991). Ionizing groups in lipopolysaccharides of Pseudomonas cepacia in relation to antibiotic resistance. Mol Microbiol. 5:641- 646.

Dijkshoorn, L.; Nemec A. and Seifert, H. (2007). An increasing threat in hospitals: multidrug-resistant Acinetobacter baumannii. Nat Rev Microbiol. 5, 939–951.

Funke, G.; Monnet, D.; deBernardis, C.; von Graevenitz, A. and Freney, J. (1998). Evaluation of the VITEK 2 system for rapid identification of medically relevant gram-negative rods. J Clin Microbiol.;36:1948-52.

Gilardi, GL. (1971).Antimicrobial susceptibility as a diagnostic aid in the identification of nonfermenting gram negative bacteria. Appl Microbial.22(5):821-3.

Gokale, SK. and Metgud, SC. (2012).Characterization and antibiotic sensitivity pattern of nonfermenting gram negative bacilli from various clinical samples in a teritiary care hospital, BELGAUM-JPBMS.;17(14).

Jamshidi, M.; Javadpour, S.;Eftekhari, TE.; Moradi, N.and Jomehpour, F. (2009). Antimicrobial resistance pattern among intensive care unit patients. Afr. J. Microbiol. Res. 3(10):590-594.

Joyanes, P.; del Carmen, Conejo M.; Martinez-Martinez L.and Perea EJ. (2001). Evaluation of the VITEK 2 system for the identification and susceptibility testing of three species of non - fermenting gram-negative rods frequently isolated from clinical samples. J Clin Microbiol.;39:3247-53.

Juyal D, Prakash R, Shanakarnarayan S.A., Sharma M, Negi V and Sharma N (2013). Prevalence of non - fermenting gram negative bacilli and their in vitro susceptibility pattern in a tertiary care hospital of Uttarakhand : A study from foothills of Himalayas. Saudi J for Health Sciences - Vol 2, Issue 2, May-Aug; 108-112.

Kirthilaxmi, K. and Benachinmardi. (2014). Prevalence of nonfermenting Gram negative bacilli and their in vitro susceptibility pattern at a teritiary care teaching Hospital. Journal of scientific society.;41(3).

Kumari, HB.; Nagaratna, S. and Chandramukhi, A. (2007 ).Antimicrobial resistance pattern among aerobic gram negative bacilli of lower respiratory tract specimens of intensive care unit patients in a neuro centre. Indian J Chest Dis Allied Sci.;49(1):19-22.

Leepethacharat, K., Oberdorfer, P. (2007). Acinetobacter baumannii infection and colonization among pediatric patients at Chiang Mai University Hospital. J Infect Dis Antimicrob Agents 24, 63–73.

Ling, T. K. W., Z. K. Liu, and A. F. B. Cheng. (2003). Evaluation of the Vitek 2 system for rapid direct identification and susceptibility testing of gram-negative bacilli from positive blood cultures. J. Clin. Microbiol. 41:4705-4707.

LiPuma, J. J.; Currie, B. J.; Lum, G. D. and Vandamme, P. A. R. (2007). Burkholderia, Stenotrophomonas, Ralstonia, Cupriavidus, Pandoraea, Brevundimonas, Comamonas, Delftia, and Acidovorax. In Manual of Clinical Microbiology, 9th edn, p.p. 749–769. Edited by P. R. Murray, E. J. Baron, J. H. Jorgensen, M. L. Landry and M. A. Pfaller. Washington, DC: American Society for Microbiology.

Manoharan, A.;Chatterjee, S.;Mathai, D.; SARI. (2010). Study Group. Detection and characterization of metallo beta lactamases producing *Pseudomonas aeruginosa*. Indian J Med Microbiol28:241-4.

O'Hara, C. M., G. L. Westbrook, and J. M. Miller. (1997). Evaluation of Vitek GNI1 and Becton Dickinson Microbiology Systems Crystal E/NF identification systems for identification of members of the family *Enterobacteriaceae* and other gram-negative, glucose-fermenting and non-glucose-fermenting bacilli. J. Clin. Microbiol. **35:**3269–3273.

Omar, N.; Abd El Raouf, H.; Okasha, H. and Nabil, N. (2015). Microbiological assessment of Burkholderia cepacia complex (Bcc) isolates in Alexandria Main University Hospital. Alexandria Journal of Medicine. 51 (1): 41–46.

Patel, PH. (2013).Prevalence of non-fermenting gram negative bacilli infection in tertiary care

Hospital in Ahmedabad, Gujarat. Indian journal of Basic Applied Medical Research. 2(6):608-13.

Pawar, M.; Mehta, Y.; Purohit, A.; Trehan, N.and Rosenthal, VD. (2008).Resistance in gram-negative bacilli in a cardiac intensive care unit in India: Risk factors and outcome. Ann Card Anaesth;11:20-6.

Rit, K.; Nag, F.; Raj, HJ. and Maity, PK. (2013). Prevalence and susceptibility profiles of Nonfermentative Gram-negative Bacilli infection in a Tertiary care Hospital of Eastern India. Indian Journal of Clinical practice. 24(5)

Robinson, A., Y. S. McCarter, and J. Tetreault. 1995. Comparison of Crystal Enteric/Nonfermenter system, API 20E system, and Vitek automicrobic system for identification of gram-negative bacilli. J. Clin. Microbiol. 33**:**364–370.

Roy, P.; Ahmed, N. H. and Grover, R. K. (2014). Antimicrobial susceptibility pattern of Burkholderia cepacia isolates from patients with malignancy. J. Global Infect Dis. 6(2): 90-91.

Simgamsetty S, Yarlagadda P, Yenigalla BM, Myneni RB.( 2016). Ease with VITEK 2 Systems, Biomerieux in Identification of Non-Lactose Fermenting Bacteria including their Antibiotic Drug Susceptibility: Our Experience. Int J Res Med Sci.;4:813-7.

Shashwati N, Kiran T, Dhanvijay AG. (2014) Study of extended spectrum b-lactamase producing *Enterobacteriaceae* and antibiotic coresistance in a tertiary care teaching hospital. J Nat Sci Biol Med.;5:30-5.

Stager, CE .and Davis, JR. (1992) Automated Systems for identification of microorganisms. Clin Microbiolol.:302-27.

Tseng, S.; Tsai1, W.; Liang, C.; Lin, Y.; Huang, J. Chung, C.; Tyan, Y. and Lu, P. (2014). The contribution of antibiotic resistance mechanisms in clinical Burkholderia cepacia complex isolates: An emphasis on efflux pump activity. PLOS ONE.www.plosone.org. 9(8): e104986.

Von Gravenitz ,A. (1973). Clinical Microbiology of unusual pseudomonas species. Prog Clin pathol.;5:185-218.