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Investigation of genes associated with Antimicrobial resistance and some virulence factors among *Burkholderia cepacia* Isolates

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ABSTRACT

Burkholderia cepacia has possesses many genes responsible for resistance to many antibiotics. This study aimed to examine and investigate genes that responsible for folate pathway antagonists, quinolones, beta-lactam drugs resistance and some virulence factors among *B.cepacia* isolated from different sourcesusing Polymerase Chain Reaction (PCR). The results showed among 950 specimens, 31 isolates were identified as *B. cepacia* isolates, and 700 (73.68%) of specimens exhibit bacterial growth compared with 250 (26.31%) no bacterial growth. Results were showed out of a total of 530 (55.78%) patients (female), 150 (15.78%) speciemen does not show any growth on the MacConkey agar , compared to 380 (40 %) speciemen that gave positive results and showed growth of bacteria. While it was from a total of 420 (44.21%) patients (male), 100 (10.52 %) speciemen does not show any bacterial growth on MacConkey agar compared with 320 (33.68 %) speciemen that gave positive results and showed bacterial growth. Results of PCR amplification showed high spreading of folic acid pathway which included *Sul-1*, *Sul-2* and *Dfr-B* which reached 20/31 (64.51%) , 28/31 (90.3%) and 1/31 (3.22%) respectively , while genes of qunolonies family appear 24/31 (77.41%) , 30/31 (96.77%) for *qnr B and Par C* respectively . The beta-lactam resistance gene such as *Ctx M* appear 15/31 (48.38%) , while that virulence factors genes involved *Stmpr-1 ,Stmpr-3, Pm1* and *OqxB* which reached 27/31 (87%) , 22/31 (70.96%) , 26/31(88. 87 %) and 31/31(100%) among *B. cepecia* isolates respectively, while *Sul-3* , *Dfr-A,qnr A ,qnr C,Stmpr-2* , *Lip* and *Qep A* were absent among *B. cepecia* isolates in this study.

Keywords*: Burkholderia cepacia*, Drugs resistance genes, Virulence factors genes.

INTRODUCTION

Burkholderia cepacia complex are at least ten closely related species combined (Genomovare) that are simplistically referred to as *Burkholderia cepacia*. It is rod-shaped, free-living, aerobic, and motile gram-negative bacteria ranging from 1.6- 3.2 μm. They have been found to possess multitrichous polar flagella as well as pili used for attachment. It is non-spore-forming straight rods with rounded ends. The colonies are circular and convex with entire margins, they grow to 1.0-1.5mm in diameter after 2days and catalase-positive (Winn *et al*.,2006 ; Tavares, *et al*., 2020)

About (30.0%) of *B. cepacia* causing burns and wounds infection as well as severe infections and suppuration and serous fluid (El-Barrawy *et al*., 2017; Chen *et al* ., 2020).People with diabetes who are at risk of developing foot ulcers.*B. cepacia* are reported to be pathogens and implicated in chronic granulomatous disease with the major site of infection being skin (Jnana, *et al*., 2020; Netten *et al*., 2020). Most *Burkholderia* contain a modified lipopolysaccharide that causes intrinsic polymyxin resistance. Contributing to reduced drug penetration are restrictive porin proteins. Efflux pumps of the resistance nodulation cell division family are major players in *Burkholderia* multidrug resistance. Altered DNA gyrase and dihydrofolate reductase targets cause fluoroquinolone and trimethoprim resistance (Rhodes *et al* ., 2016).Several mechanisms contribute to the bacteria's pan resistance, including inducible chromosomal-lactamases, altered penicillin-binding proteins, restricted membrane permeability, function of porins, drug target modifications, presence of multiple multidrug efflux pumps confer resistance to quinolones, chloramphenicol and trimethoprim. Some strains have inducible β-lactamases or dihydrofolate reductases and changes in lipopolysaccharide structure. The resistance mechanism is complex and is thought to be partially attributable to a unique LPS structure (CLSI., 2020).Some of the potential virulence factors currently under investigation are the cable pili, protease, lipase, phospholipase C (PLC), hemolysin, a melanin-like pigment, flagella, and the siderophores salicylic acid, pyochelin, cepabactin, and ornibactin.

B. cepacia produces a heat-labile haemolysin which has both phospholipase C and sphingomyelinase activities (Mandell *et al*., 2020).

MATERIALS AND METHODS

Patients and Specimens Processing

Present study included 950 obtained from different clinical source randomly for patients suffering different diseases included Burn, wound, diabetes foot ulcer, urine, throat and tissue were attended to main hospitals and burn center inboth Al-Najaf City and Baghdad City /Iraq, as well as clinical laboratories in Al-Najaf City-Iraq within the period several months started in from February to September 2022. Using swabs all specimens were streaked on MacConkey agar and OFPBL agar and incubated aerobically in 37^{o}C overnight under sterile conditions (Collee *et al*.,1996).

Diagnosis of *B. cepacia* Isolates

Every suspected gram-negative bacteria which have features of *B.cepacia*, based on microscopic , morphological characteristics, oxidase, as well as some main biochemical tests and then streaked on MacConkey Agar and OFPBL agar (MacFaddin, 2000;Naveen etal.,2019). Monoplex PCR using to detect *Burkholderia* species via specific primer (*Bur S*). The final identification was performed using the automated Vitek-2 compact system using ID-GP cards.

DNA extraction and PCR assay

Following the instructions provided by a manufacturing company, a genomic DNA extraction micro kit (Favorgen, South Korea) was used to collect all of the nucleic acid for 31 clinical isolates of *B. cepacia*. This was done in accordance with the manufacturer's protocol. After ensuring the integrity of the whole DNA sample by storing it in a deep freezer set to -20 degrees Celsius, a PCR analysis was carried out in order to test for the genes listed in Table 1. The equipment for gel documentation was employed for the migration of PCR amplification (bands) at 1% agarose, and then the bands were dyed with Nuclic Acid Stain at a concentration of 0.5 g/ml thereafter.

Table (1): Primer Sequence and condition

Gene name	Primer Sequence 5' to 3'	Annealing (°C)	Size of product (bp)	Reference
Sul-1-F	GTGACGGTGTTCGGCATTCT	54.7	921 bp	(Lanz *et al*., 2003)
Sul-1-R	TCCGAGAAGGTGATTGCGCT			
Sul-2-F	CGGCATCGTCAACATAACCT	51.5	721 bp	(Boerlin *et al*., 2005)
Sul-2-R	TGTGCGGATGAAGTCAGCTC			
Sul-3-F	CAGATAAGGCAATTGAGCATGCTC TGC	55	569 bp	(El-Kazzaz *et al*., 2016)
Sul3-R	GATTTCCGTGACACTGCAATCATT			
DfrA-F	CACTTGTAATGGCACGGAAA	57	270 bp	(Coelho, 2016)
DfrA-R	CGAATGTGTATGGTGGAAAG			
DfrB-F	AATTGTGTTAAATTAAAGATAACTT	43	572 bp	(Coelho, 2016)
DfrB-R	TAAGTATTCTTTAGATAAATCGGAT			
qnrA –F	GATAAAGTTTTTCAGCAAGAGG	57	593 bp	(Jacoby *et al.*, 2003)
qnrA –R	ATCCAGATCGGCAAAGGTTA			
qnrB –F	ATGACGCCATTACTGTATAA		560 bp	

qnrB –R	GATCGCAATGTGTGAAGTTT	53		(Jacoby *et al.*, 2006)
qnrC-F	GGGTTGTACATTTATTGAATC	50	447 bp	(Wang *et al.*, 2009)
qnrC-R	TCCACTTTACGAGGTTCT			
Par C-F	CTGAATGCCAGCGCCAAATT	56	389 bp	Iranzad *et al* .,2017)(
Par C-R	TGCGGTGGAATATCGGTCGC			
Ctx M-F	AACCGTCACGCTGTTGTTAG	57	766 bp	(Newire *et al.*, 2013)
Ctx M-R	TTGAGGCGTGGTGAAGTAAG			
Stmpr-1-F	CAACGACTCGATGAATGTGG	52	174 bp	(Molloy *et al.*, 2019)
Stmpr-1-R	CAGACATAGCCGTTCGGATT			
Stmpr-2-F	CAGGTCGAGAGCATCATCAA	55	168 bp	(Molloy *et al.*, 2019)
Stmpr-2-R	GGTCACCGGTACGTTGTTCT			
Stmpr-3-F	AGCGAAAACACGATTCGTTC	55	189 bP	(Molloy *et al.*, 2019)
Stmpr-3-R	ACGGTGATGACGTTGAACAG			
Lip-F	CAGGCCTACAAGCTGCACTA	60	326 bp	(NCBI)
Lip-R	TTGACGAGGTCGATGGCATT			
Pm1-F	GGATCATCTATAATGAAACTG	40	563 bp	(Sosa *et al* ., 2006)
Pm1-R	CTGATAATCAACTTGGAAGTT			
Oqx B-F	CGAAGAAAGACCTCCCTACCC	62	240 bp	(Chen *et al* ., 2012)
Oqx B-R	CGCCGCCAATGAGATACA			
Qep A-F	CTGCAGGTACTGCGTCATG	60	403 bp	(Cattior *et al.*, 2008)
Qep A-R	CGTGTTGCTGGAGTTCTTC			

RESULTS AND DISCUSSION

Patients and bacterial growth

Results of this study showed among 950 non-duplicated patient was 700 (73.68%) bacterial growth compared with 250 (26.31%) no bacterial growth.The results of biochemical tests,Vitek-2 system and PCR showed among 950 specimens, 31isolates were identified as *B. cepacia* isolates.

B.cepacia isolates were mostly observed from burns 15 (3.33%) , while the ratio in wounds 5(3.33%) and 5 (3.84%) for diabetic foot,3(2.4%) for urine speciemes and 2 (2.15%) for throat swabs and 1 (50%) for tissue speciemens .

Table (2): Distribution of *B.cepacia* on the MacConky agar depending on the differential source of infection

Sample source	Totale	growth	No growth	Total of*B.cepacia*
Burn	**450**	**320**	**130**	**15 (3.33%)**
Wound swap	**150**	**109**	**41**	**5 (3.33%)**

Sample source	Totale	growth	No growth	Total of*B.cepacia*
Diabates feet	**130**	**85**	**45**	**5 (3.84%)**
Urine	**125**	**105**	**20**	**3 (2.4%)**
Throat swap	**93**	**80**	**13**	**2 (2.15%)**
Tissue	**2**	**1**	**1**	**1 (50%)**
Total	**950**	**700**	**250**	**31 (3.26 %)**

Detection of folate pathway inhibitors genes among *B. cepecia* isolates

Results of PCR amplification showed high spreading of folic acid pathway which included *Sul-1, Sul-2* and *Dfr-B* which reached 20/31 (64.51%) , 28/31 (90.3%) and 1/31 (3.22%) respectively , while *Sul-3* , *Dfr-A* absent among *B. cepecia* isolates and not detect in present study. Another study a comparative with present study, 501 isolates of *E.coli* it was studied on the sulfonamide resistance genes, the study showed that the bacterial isolates were carrying the genes of *Sul-2, Sul-1*, and *Sul-3* a ratio 65% , 45% and 12% respectively (Wu *et al* ., 2010).

Another local study in Najaf city , 28 of *Acinetobacter* spp isolates . The study showed that the bacterium was carrying a gene of *Sul-1* a ratio (100 %),while a ratio 89.29% and 75% for the *Sul-2* and *Dfr-A* genes respectively, while *Sul-3* and *Dfr -B* genes did not give any positive result (Naseer*et al* ., 2022).

Detection of Qunolonies family genes among *B. cepecia* isolates

Results of PCR amplification genes of qunolonies family showed 24/31 (77.41%) ,30/31(96.77%) for *qnr B and Par C* respectively, while *qnr A* and *qnr C* genes absent among *B. cepecia* isolates and not detect in current study. A local study in Najaf on bacterial isolates, out of a total of 22 isolates, the study showed that 5/22(8.33%) of *E. coli* possessed *qnr B,* also another study showed that among 107 *E. coli* and *Klebsiella* isolates,74/107 (47.74%) carried the *qnr B* (Salah *et al* ., 2019 ; *Samer et al* ., 2021). Another study around *Par C* gene in Iran with *E.coli* Isolates from Urinary Tract Infections , the study showed the frequency of intact genes among ciprofloxacin–resistant isolates was 90.9% for *parC* as in (Iranzad*et al*., 2017),while another study around *S.typhi* isolates showed about 26/28 (92.85 %)from isolates carried *Par C* gene (Nathania *et al*., 2022).

Detection of some beta-lactam resistance genes among *B. cepecia* isolates

Results of PCR amplification genes of beta-lactam such as *Ctx M* showed15/31 (48.38%) in current study.A local study was conducted in Najaf , 16/30 (53.33%) isolates of *B.cepacia* that carried *Ctx M* resistance gene (Alnasrawy *et al* ., 2021).One of the major leading causes of resistance among Gram-negative bacteria to ß-lactam antibiotics is ESBLs. These enzymes are plasmid-encoded β-lactamases that mediate resistance to cephalosporins of the first, second and third generations, such as cefotaxime, ceftriaxone and ceftazidime, to penicillins. The main genetic classes of ESBLs among the clinically relevant Gram-negative bacteria is *CTX-M* (Elsafi, 2020 ; Khan,*et al*.,2020).

Detection of virulence factors genes among *B. cepecia* isolates

Results of PCR amplification genes ofvirulence factors involved *Stmpr-1 ,Stmpr-3, Pm1* and *OqxB* which reached 27/31 (87%) , 22/31 (70.96%) , 26/31(88. 87 %) and 31/31(100%) among *B. cepecia* isolates respectively, while *Stmpr-2 , Lip* and *Qep A* were absent among *B. cepecia* isolates in this study.Other studies were conducted on *Stenotrophomona maltophilia,* and all the isolates carried the *Stmpr*-1gene by 100% as in (Fluit *et al*. (2021), while the Elbaradei & Yakout (2022) study showed that the bacterial isolates carried 70% and 15% of the genes *Stmpr*-1 and *Stmpr*-2 respectively , and this is consistent with the molecular results of our study except *Stmpr*-2 gene.Another study conducted in Jordan among genes of virulence factors of *Proteus mirabilis,* the study showed that 63 isolates out of 58/63 (92.1%) carrying a

positive result for *Pm1* gene as in study (Emad *et al*., 2020), and this is consistent with the molecular results of present study. Another study conducted in China in 2012 , among the 1022 *E.coli* isolates showed 206 (20.2 %) that carried *OqxB* gene as in (Chen *et al* ., 2012) , while study conducted in Iran, showed 90/100 (90 %) of *K.pneumoniae* that encod and carried *Oqx B* efflux pumps gene (Amereh *et al* ., 2023). However , the genes that used in current study and distribution of *B. cepacia* isolates for these genes they are showed in the Table (3) .

Table (3): Distribution of *B. cepacia* isolates for genes used in current study.

No	*Sul-1*	*Sul-2*	*DfrB*	*qnr-B*	*Par C*	*CTX-M*	*Stmpr-1*	*Stmpr-3*	*Pm 1*	*Oqx B*
1	-	+	-	-	+	+	-	-	+	+
2	+	+	-	+	+	+	+	-	+	+
3	+	+	-	+	+	-	+	+	+	+
4	-	-	-	+	+	-	+	+	+	+
5	+	+	-	+	+	+	+	+	+	+
6	+	+	-	-	+	-	+	+	+	+
7	-	-	-	-	+	+	+	+	+	+
8	+	+	-	+	+	+	+	-	+	+
9	+	+	-	+	+	-	+	+	+	+
10	-	-	-	+	+	-	+	+	+	+
11	-	-	-	+	+	-	+	-	+	+
12	-	-	-	-	+	-	+	-	+	+
13	+	+	-	+	+	+	+	-	+	+
14	+	+	-	+	+	-	-	+	+	+
15	+	+	-	+	+	+	+	+	+	+
16	+	+	-	+	+	-	+	+	+	+
17	+	+	-	+	+	-	+	+	+	+
18	+	+	-	+	+	-	+	+	+	+
19	+	+	-	+	+	+	+	+	+	+
20	+	+	+	+	-	-	+	+	+	+
21	+	+	-	+	+	+	+	+	+	+
22	-	+	-	+	+	+	+	+	+	+
23	+	+	-	+	+	+	-	-	-	+
24	-	+	-	+	+	-	-	-	-	+
25	+	+	-	+	+	+	+	+	-	+
26	+	+	-	+	+	+	+	+	-	+
27	-	+	-	+	+	+	+	+	-	+
28	+	+	-	+	+	+	+	+	+	+
29	+	+	-	-	+	-	+	+	+	+
30	-	+	-	-	+	-	+	+	+	+
31	-	+	-	-	+	-	+	-	+	+

REFERENCES

Al-ghazaly, N., Tuwaij, N.(2022). Genetic Detection of Folate Pathway Inhibitors Genes among *Acinetobacter spp*. Isolates. *The Egyptian Journal of Hospital Medicine*. 88, 3537-3542.

Alnasrawy, W., & AL-Aammar, M.(2021). A Molecular Study with A Comparison of the Odds of Diagnostic Methods For Burkholderia Cepacia Bacteria Isolated from Patients with Diabetic Foot Ulcer. *Indian Journal of Forensic Medicine & Toxicology*, *15*(3), 4603.

Amereh, F., Arabestani , M., Hosseini, S., Shokoohizadeh, L.(2023).Association of qnr Genes and OqxAB Efflux Pump inFluoroquinolone-Resistant *Klebsiella pneumoniae* Strains. *Int J Microbiol*. 2023: 9199108.

Boerlin, P.,Travis, R, Gyles, C., Reid-Smith,R., Lim, N., Nicholson,V.,McEwen, S., Friendship, R., Archambault, M .(2005). Antimicrobialresistance and virulence genes of Escherichia coli isolates from swine in Ontario. *Applied and Environmental Microbiology*. 71(11) : 6753-6761.

Cattoir, V., Poirel , L., Nordmann, P. (2008). Plasmid -mediated quinolone resistance pump *Qep A2* in an *E.coli* isolate from France. Anti-microb. *Agents Chemother*. 52 : 3801-3804.

Chen, Y. *et al*. (2020).Trends in microbial profle of burn patients following an event of dust explosion at a tertiary medical center. *BMC Infect. Dis.* 20: 193.

Chen, X., Zhang, W., Pan , W., Yin , J., Pan, Z., Gao , S., Jiao , X. (2012). Prevalence of *qnr*, *aac*(6 ')-*Ib-cr*, *qepA*, and *oqxAB* in *Escherichia coli* Isolates from Humans, Animals, and the Environment. *Antimicrobial Agents and Chemotherapy* . 56 (6) : 3423–3427.

CLSI . (2021). Performance standards for antimicrobial susceptibility testing. 30th edition. CLSI guideline M100. Wayne, PA: *Clinical and Laboratory Standards Institute*.

Coelho, C. (2016): Trimethoprim-sulfamethoxazole resistance in *Staphylococcus aureus* in Africa: Distribution of resistance genes and evaluation of the success of major MRSA clones (Doctoral dissertation). https://biblios.ciencias.ulisboa.pt › detalhes › 42253?lang=eng

Collee, J. G., Mackie, T. J., & McCartney, J. E. (1996). *Mackie & McCartney practical medical microbiology*. Harcourt Health Sciences.

El-Barrawy, M., Mohamed, O., Behery, A. and Hazzah ,W.(2017). Existence of *Burkholderia cepacia* at Burn and Intensive Care Units in Alexandria Main University Hospital, Egypt , Asmaa A. Hendawy *JMSCR*, 05 (12): 31376-31385.

El-Kazzaz ,S., Mashaly, G., El-Sabbagh, A., Abd Elmagid, D.(2016). Resistance genes to sulphonamide in commensal *Escherichia coli* isolated from stool of patients in Mansoura University Children Hospital. *African Journal of Microbiology Research.* 10 (33):1363-1370.

Elsafi**,** S.(2020). Occurrence and Characteristics of the Extended-spectrum Beta-lactamaseproducing Enterobacterale in a Hospital Setting . *The Ope Microbiology Journal*, 14:190-195.

Iranzad, M., Hakemi-Vala, M. (2017). The Role of *parC*, *parE*, and *qnrB* Genes in Ciprofloxacin-Resistant *Escherichia coli* Isolates from Urinary Tract Infections. *Arch Pediatr Infect Dis*. 5 (3) : e41504.

Jacoby,G ., Chow, N and Waites, K . (2003). Prevalence of plasmid-mediated quinolone resistance. *Antimicrob Agents Chemother*. 47(2): 559–562.

Jacoby, G.,Walsh, K.,Mills, D.,Walker, V.,Oh, H., Robicsek, A. and Hooper D.(2006). Qnr B,another plasmid-mediated gene for quinolone resistance. *Antimicrob Agents Chemother*. 50(4):1178-1182.

Jnana , A., Muthuraman, V., Varghese, V.,Chakrabarty, S. , Murali, T., *et al*. (2020) . Microbial community distribution and core microbiome in successive wound grades of individuals with diabetic foot ulcers. *Appl.Environ. Microbiol.* 86: 2608-19.

Khan**,** M.;Thurgood, N.; Faheem, S.; Rais,N.; Ansari, M.; Sultan,M and Shams,T.(2020). Occurrence of Extended Spectrum Beta-Lactamase Gram-Negative Bacteria from Non-Clinical Sources in Dubai, United Arab Emirates. *Water*, 12:2562.

Lanz, R., Kuhnert, P., Boerlin, P. (2003). Antimicrobial resistance andresistance gene determinants in clinical Escherichia coli from different animal species in Switzerland. *Veterinary Microbiology.* 91(1):73-84.

MacFaddin, J.F. (2000) Biochemical Tests for Identification of Medical Bacteria. 3rd Edition, Lippincott Williams & Wilkins, Philadelphia.

Mandell, D., and Bennett's principles and practice of infectious diseases "Cystic Fibrosis". John ,E., Bennett, R., Martin ,J. Blaser (9th ed.) .(2020). *Philadelphia, PA*. p. 954.

Molloy, K., Stephen,G., Smith, S., Cagney, G., Dillon, E., Greene, C., & Mcelvaney, N. (2019). Characterisation of the Major Extracellular Proteases of *Stenotrophomoas maltophilia* and Their Effects on Pulmonary Antiproteases. *Pathogenes* . 8 (92) : 1–20.

Nathania ,I., Nainggolan, I., Yasmon, A., Nusatia, A., Tjoa, E., Gunardi, W., Lucky,H. (2022). Hotspots sequences of *gyrA, gyrB, parC,* and *parE* genes encoded for fluo, roquinolones resistance from local *Salmonella Typhi* strains in Jakarta. *BMC Microbiology* .22: 250 .

Naveen , KD., Balaji,V.(2019). Accurate identification and epidemiological characterization of *Burkholderia cepacia* complex: an update. *Annals of Clinical Microbiology and Antimicrobials*.18(7) : 12-14.

Netten**,** J., Bus, S., Apelqvist, J., Lipsky, B., Hinchliffe, R., *et al.*(2020). Definitions and criteria for diabetic foot disease . *Diabetes Metab Res Rev*.

Rhodes, K., Schweizer, H.(2016). Antibiotic resistance in *Burkholderia* species. *Drug Resist. Update* . 28: 82–90.

Salah,F., Soubeiga , S., Ouattara , A., Sadji , A.(2019). Distribution of quinolone resistance gene qnr) in ESBL-producing *Escherichia coli* and*Klebsiella* spp. in Lomé, Togo. *Antimicrobial Resistance and Infection Control* , 8:104.

Samer, A., Zainab, J., Kreem ,G.(2021). Prevalence of Plasmid-mediated quinolone resistance genes among Ciprofloxacin-nonsusceptible *Escherichia coli* and *Klebsiella pneumoniae* isolated from clinical isolates in Najaf, Iraq. *Journal of Pharmacy and Technology*, 14:4.

Sosa , V., Schlapp, G. and Zunino, P. (2006). *Proteus mirabilis* isolates of different origins do not show correlation with virulence attributes and can colonize the urinary tract of mice . *Microbiology*. 152: 2149–2157.

Tavares , M. ; Kozak, M. ; Balola, A. ; Coutinho, C.P. ; Godinho, C.P. ; Hassan, A. A. ; Cooper, V.S. and Sá-Correia, I. (2020). Adaptation and Survival of *Burkholderia cepacia* and *B. contaminans* During Long-Term Incubation in Saline Solutions Containing Benzalkonium Chloride. *Front. Bioeng. Biotechnol.* 8:630.

Tuwaij, N., Aziz , Z., Al-ghazaly, N.(2020). Investigation of genes associated with producing betalactamase among *Burkholderia cepacia* isolates. *Eurasia J Biosci* 14, 1919-1924.

Wang, M.,*et al***.** (2009). New plasmid-mediated quinolone resistance gene, *qnrC*, found in a clinical isolate of *Proteus mirabilis*. *Antimicrob. AgentsChemother*. 53 (5) :1892–1897.

Winn, W,, Allen, S., Janda, W., Koneman, E., Procop, G., Schreckenberger, P., *et al*. (2006). Editors. Nonfermenting Gram negative bacilli. In: Koneman‟sColor Atlas andtextbook of diagnostic Microbiology, 6th ed. USA: *Lippincott Williams and Wilkins Company* . p. 305-391.

Wu ,S., Dalsgaard ,A., Hammerum ,A., Porsbo, L., Jensen ,L.(2010). Prevalence and characterization of plasmids carrying sulfonamide resistance genes among Escherichia coli from pigs, pig carcasses and human. *Acta Vet Scand*. 52:47.