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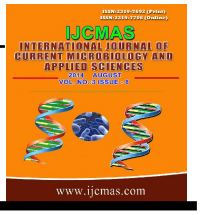

Original Research Article

Detection of Biofilm Formed by *E.coli* Isolated from various animal diseases and evaluate it's protective role

Hassan A. Abdul-Ratha[1*] and Ban N. Nadhom[2]

[1]University of Baghdad/College of Agriculture, Iraq
[2]University of Baghdad / College of Veterinary medicine, Iraq
**Corresponding author*

A B S T R A C T

In this study, 284 samples were collected (182 samples from case of mastitic milk and 102 fecal samples) from Salahaddin governorate, College of veterinary medicine-university of Baghdad, College of Agriculture-university of Baghdad, Radhwanya zoon, Dora zoon and Abu-Ghraib zoon, samples were cultured on MacConkey agar and Eosin Methylene Blue agar, after purification of cultured bacteria, biochemical tests, API 20 E System and RapID^{TM} ONE System kit were done, results showed that 54 out of 182 milk samples were positive for *E.coli* and 91out of 102 fecal samples were positive for*E.coli*,the ability of these *E.coli*isolates to produce biofilm were detected and the results showed that 50 out of 54 The resistance of these *E.coli*isolates against (10) of antibiotics (Bacitracin, Chloramphenicol, Imipenem, Vancomycin, Gentamycin, Ciprofloxacin, Nalidixic acid, Amoxycillin, Ceftriaxone and Tobramycin) were studied and results showed that all these isolates were resistant (100%) toAmoxycillin, Vancomycin and Bacitracin in addition the results showed that the resistance of biofilm producer isolates and non-biofilm producer isolates against these antibiotics were differed , biofilm producer isolates were more resistant than group of non-biofilm producer isolates against all (10) of antibiotics except Ceftriaxone at which group of non producer biofilm was more resistant than group of producer biofilm.

Keywords

Biofilm;
E.coli;
Antibiotics;
Mastitis;
Diarrhea

Introduction

*E.coli*is a normal inhabitant of the intestines of most animals, including humans. Some *E.coli* strains can cause a wide variety of intestinal and extra–intestinal diseases, such as diarrhea, urinary tract infections,septicemia, mastitis and neonatal meningitis. (Tenaillon et al., 2010).The formation of bacterial biofilms of *E.coli* in a host in general seems to be, based on current evidence, to a large extent an intra cellular event (Anderson *et al*.2010).

The diseases caused by a particular

strain of *E. coli* depend on distribution and expression of many virulence determinants such as adhesion ,biofilm formation, production of haemolysin, enterotoxin, shiga toxin ,endotoxin and capsules formation (Kaper et al ., 2004).

Biofilms are not easily defined as they vary greatly in structure and composition from one environmental niche to another,,Microbial biofilms are extremely complex microbial ecosystems consisting of microorganisms attached to a surface and embedded in an organic polymer matrix of microbial origin, As well as microbial components, non–cellular materials such as mineral crystals, corrosion particles, clay or silt particles, or blood components, may also be found in the biofilm matrix, Biofilms, particularly in water systems,can be highly complex, whilst others such as those on medical devices, may be simpler, and composed of single, cocciod or rod shaped organisms, Given these differences, it does not seem plausible to suggest that a true "biofilm model" can be defined that is applicable to every ecologicalindustrial and medical situation, Therefore the definition of a biofilm has to be kept general and thus may be redefined as" microbial cells immobilized in a matrix of extra cellular polymers acting as an independent functioning ecosystem, homeostatic ally regulated" (Percival et al . 2000).

Numerous authors state that bacteria in a biofilm are 10 to 1000-fold more resistant to antibiotics than in their planktonic form (Gorski and Palmer,2007; Melchior et al., 2006). This study aimed to isolate *E.coli* from mastitis cases (milk samples) and diarrheal cases (fecal samples) and detect their ability to produce biofilm and it's resistant to antibiotics.

Materials and Methods

MacConkey agar and Eosin Methylene Blue agar were used for growth and isolation of *E.coli* from 182 milk and 102 fecal samples "Total 284 samples" which were collected from Salahaddin governorate, Abu- Ghraib zoon, College of veterinary medicine - university of Baghdad, College of agriculture - university of Baghdad, Radhwanya zoon and Dora zoon from cows suffering from mastitis and apparently normal cows in case of milk samples and from animals (sheep, goats, cows and calves) suffering from diarrhea in case of fecal samples. Morphological, cultural and biochemical tests in addition to API20 E system and RapID^{TM} ONE System.System were used for the diagnosis of these isolates, A qualitative assessment of biofilm formation was determined by tube method (Christensen et al., 1982).

Antibiotic susceptibility test was performed using a disc diffusion method on Mueller – Hinton Agar according to Bauer –Kirby et al., 1966, discs of antibiotics used were: Bacitracin, Chloramphenicol, Imipenem, Vancomycin, Gentamycin, Ciprofloxacin, Nalidixic acid, Amoxicillin, Ceftriaxone and Tobramycin.

Results and Discussion

Results of bacterial isolation showed that out of total 182 milk samples were collected from cows suffering from acute mastitis (104 samples) and apparently normal cows

(78 samples), 54 milk samples (29.67%) showed positive results for the presence of *Escherichia coli* after culturing one EMB agar. Sara., 2014. Found that 31 out of 110 milk samples collected from cows infected with acute and subclinical mastitis of cows distributed between Baghdad and Diyala governorate showed positive results for *E.coli* (28.18%) and this is in agreement with our results.

Results in table (1) showed the differences in number of positive isolation according to the region from which samples were collected.

On the other hand out of 102 fecal samples (52 fecal samples from cows and 50 fecal samples from sheep), 91 fecal samples (89.21%) showed positive results for the presence of *E.coli* after culturing on EMB,Chapman *et al*. (1994) were able to detecte*E.coli* O157: H7 in 84 (8.2%) of 1024 bovine rectal swab, whereas direct plating on SMAC agar detected only 23 positive cases (2.2%). Other survey showed that using a selective enrichment step increased the recovery (by almost twofold) of *E.coli* O157: H7 from bovine stool specimens (Zhao *et al*., 1995).the results in the table (2) showed the difference in number of positive isolation according the region from which samples were collected .

The results of samples culturing showed different morphological characteristics of *E.coli* on different media, after incubation at37°C for 24hours. On MacConkey agar colonies were appeared as red/pink color while on Eosin Methylene Blue EMB agar the visible colonies were appeared as green metallic sheen this results agree with (Quinn et al., 2004). Isolated bacteria were appeared as gram negative rods under light microscopic lense like (Schulze *et al*., 2006). The red /pink color on MacConkey agar was occurred due to the utilization of the lactose that available in the medium with surrounding areas of precipitated bile salts, While Eosin Methylene Blue EMB agar was used for selection and isolation purposes, and was considered as a rapid and accurate method for distinguishing *E. coli* from other gram-negative pathogens. The visible colonies were appeared as green metallic sheen that indicating vigorous fermentation of lactose and acid production which precipitates the green metallic pigment .This result agree with (Quinn *et al* ., 2004). The Biochemical identification of the bacterium has showed that this bacterium was Catalase positive, oxidase negative, lactose fermentive, not produce H_2S, Indole positive, motile, have ability to gas production, citrate utilization negative Kligler test negative and Urease negative (Table. 3) to confirm the diagnosis, Api 20 E system and RapID^{TM} ONE System were used . Results confirmed that 91 isolates out of 102 fecal samples were belong to *E.coli* and 54 isolates out of 182 milk samples were belong to *E.coli.*

Detecting the ability of *E.coli* isolates to produce biofilm The test was done by using Christensen tube method (Christensen *et al*.,1982). the results showed that 50 out of 54 *E.coli* isolates from milk samples produced biofilm (92.59%),38 out of 49 *E.coli* isolates from fecal samples of cow produce biofilm (77.55%)and 39 out of 42 *E.coli* isolates from fecal samples of sheep produce biofilm (92.85%) ,Mae da (2013) showed that 46 of total 56 isolate of *Staphylococcus aureus* produced biofilm (82.14%), Vasudevan et al ., (2003) who demonstrated that 32 of 35 *S. aureus* isolates were produced biofilm (91.42) these results in agreement with our results while Baselga et al .(1993) found a lower percentage (12%) of biofilm-positive producer strains in 92 bovine strains tested, table (4,5,6)while 4

isolates from milk samples,11 isolates from 49 fecal samples of cow and calves and 39isolates from 42 fecal samples of sheep had not produce biofilm. The thickness of biofilm ranged between (0.2-2)mm, Al-Ithawy (2010) found that the thickness of biofilm produced *pseudomonas aeruginosa* ranged between (1.1-6.5)mm while Mae da (2013) showed that the thickness of biofilm produced *S.aureus* ranged between (0.2-1.5)mm and this is in agreement with our results ,table (7,8,9). Sensitivity test of (16) biofilm producer isolates against (10) of antibiotics showed that all these isolates were resistant 100% to Bacitracin, Vancomycin and Amoxycillin. and resistant to other antibiotics at various ratio as illustrated in Table(10) .

On the other hand , Sensitivity test of (6) non biofilm producer isolates against (10) of antibiotics showed that all these isolates were susceptible 100% to Imipenem, Gentamicin, Ciprofloxacin and Chloramphenicol and Tobramycin (67%) and resistant 100% to Bacitracin, Vancomycin and Amoxycillin, these isolates also showed resistant to Ceftriaxone (83%) as showed in the table (11).

Nickel *et al*., 1985; Mah and O'toole, 2001 have shown that 10 - 1000 times more antibiotics are required to treat an infection caused by a biofilm-associated organism than a planktonic microbe of the same species. Biofilms also provide an ideal niche for the exchange of extra chromosomal DNA responsible for antibiotic resistance, making it a perfect milieu for emergence of drug resistant pathogens (Donlan and Costerton , 2002).

E.coli has often higher degrees of antimicrobials resistance which have a long history of use (Alhaj*etal*, .2007). Series of studies on the resistance of *E. coli* which were isolated from animals and humans strongly suggested that those bacteria which are resistant to antimicrobials used in animals would also be resistant toantimicrobials used in humans (WSPA, 2006; Miles *et al*., 2006;Umolu *et al*., 2006).The results of inhibitory zone diameter indicated the sensitivity of *E.coli* after (24hrs.) of incubation, towards different antibacterial concentrations, all antibacterial activities were observed to be concentration dependent that is in agreement with Clinical andLaboratory Standards Institute,(2006).

The vast majority of microbes grow as biofilm in aqueous environments; these biofilms can be benign or pathogenic, releasing harmful products and toxins, which become encased within the biofilm matrix, Biofilm formation is a phenomenon that occurs in both natural and in man- made environments under divers conditions , occurring on most moist surfaces , plant roots and nearly every living animal . (Percival et al., 2011).

Studies have shown (Nickel *et al*., 1985; Mah and O'toole, 2001) that 10 - 1000 times more antibiotics are required to treat an infection caused by a biofilm-associated organism than a planktonic microbe of thesame species. Biofilms also provide an ideal niche for the exchange of extra chromosomal DNA responsible for antibiotic resistance, making it a perfect milieu for emergence of drug resistant pathogens (Donlan, 2002). Generally, much of biofilm-associated antibiotic resistance observed in this study can be attributed to:
The extra cellular polymeric substances (EPS) secreted by biofilm bacteria, acts as a physical/chemical barrier, thus preventing penetration by many antibiotics (Thien and O'toole, 2001; Jefferson *et al*., 2005).

Table.1 Results of isolation of *E.coli* from milk samples

City of collected samples	Total No.	Number of acute mastitis	Number of apparently normal cows	Positive samples on	
				MacConkey agar	EMB agar
Salahadd in Governoratete	105	77	28	56	31
College of veterinary-medicine university of Baghdad	10	2	8	7	2
College of agriculture-university of Baghdad	40	3	37	24	3
Radhwanya zoon	20	16	4	18	16
Abu- Ghraib zoon	7	6	1	5	2
Total No.	182	104	78	110	54

Table.2 Results of isolation of E.coli from fecal samples

city of collected samples	Total No.	Positive samples on	
		MacConkey agar	EMB agar
Abu- Ghraib zoon	35	33	32
College of veterinary-medicine university of Baghdad	28	26	23
College of agriculture-university of Baghdad	17	16	16
Dora zoon	22	20	20
Total No.	102	95	91

Table.3 Results of cultural, Microscopical, and biochemical tests

Biochemical test	Result
Catalase test	(+) ve
Oxidase test	(-) ve
simmon citrate test	(-) ve
Urease test	Yellow/Yellow with gas production
Kligler Iron test	(+) ve
Indol test	(+) ve
Motility test	(+)ve
Methyl red test	(+) ve
Vogas–proskaure test	(-) ve
Gram stain	(-) ve
Cell morphology	Smooth, glassy and translucent, rosy pink on MacConkey, with the appearance of metallic sheen when growing on Eosin methylene blue .

Table.4 Results of biofilm production of different *E.coli*isolates from milk samples:

No. of isolates	Result	No. of isolates	Result	No. ofisolates	Result	No.of isolates	Result
1	+	15	+	29	+	43	+
2	-	16	+	30	+	44	+
3	+	17	+	31	+	45	+
4	+	18	+	32	+	46	+
5	+	19	+	33	+	47	+
6	+	20	+	34	+	48	+
7	+	21	+	35	-	49	+
8	+	22	+	36	+	50	+
9	+	23	+	37	+	51	+
10	+	24	+	38	+	52	-
11	+	25	+	39	+	53	+
12	+	26	+	40	+	54	+
13	+	27	-	41	+		
14	+	28	+	42	+		

Table.5 Results of biofilm production of different *E.coli*isolates fromfecal samples (cow):

No. of isolates	Result	No. of isolates	Result	No. of isolates	Result	No.of isolates	Result
1	-	14	-	27	+	40	+
2	+	15	+	28	+	41	+
3	+	16	+	29	+	42	-
4	-	17	+	30	+	43	+
5	+	18	+	31	+	44	+
6	+	19	+	32	+	45	+
7	+	20	+	33	+	46	+
8	+	21	+	34	+	47	+
9	-	22	-	35	+	48	-
10	+	23	+	36	+	49	-
11	-	24	+	37	-		
12	+	25	+	38	+		
13	+	26	+	39	-		

Table.6 Results of biofilm production of different *E.coli* isolates from fecal samples sheep

No. of isolates	Result	No. of isolates	Result	No. of isolates	Result	No.of isolates	Result
1	+	12	+	23	+	34	+
2	+	13	+	24	+	35	+
3	+	14	+	25	+	36	+
4	+	15	+	26	+	37	+
5	+	16	+	27	+	38	+
6	+	17	-	28	+	39	+
7	-	18	+	29	+	40	+
8	-	19	+	30	+	41	+
9	+	20	+	31	+	42	+
10	+	21	+	32	+		
11	+	22	+	33	+		

+ produce biofilm - not produce biofilm

Table.7 Thickness of biofilm produced by *E.coli*isolates from milk samples

No. of isolate	Thickness of biofilm (mm)	No. of isolate	Thickness of biofilm (mm)
1	0.6	28	0.2
3	1	29	1
4	0.3	30	1
5	0.2	31	2
6	1	32	1.5
7	0.2	33	0.2
8	0.7	34	0.3
9	0.2	36	1
10	1.5	37	0.4
11	0.2	38	0.2
12	0.5	39	0.6
13	1.5	40	1
14	0.4	41	0.2
15	0.7	42	0.8
16	2	43	0.2
17	1	44	0.2
18	0.4	45	0.6
19	0.3	46	0.8
20	0.2	47	0.2
21	0.6	48	0.2
22	2	49	1
23	1.5	50	0.4
24	0.2	51	0.3
25	0.2	53	0.6
26	0.2	54	0.2

Table.8 Thickness of biofilm produced by *E.coli* isolates from fecal sample of cows

No. of isolate	Thickness of biofilm (mm	No. of isolate	Thickness of biofilm (mm
2	0.2	26	0.2
3	2	27	0.2
5	0.7	28	0.2
6	1.5	29	0.5
7	0.8	30	1
8	0.2	31	0.9
10	0.2	32	0.2
12	1	33	1
13	0.4	34	0.5
15	0.2	35	0.2
16	0.2	36	0.9
17	1.5	38	0.5
18	1	40	0.5
19	1	41	0.6
20	0.4	43	0.5
21	0.5	44	0.3
23	0.3	45	0.7
24	0.5	46	0.2
25	2	47	0.4

Table.9 Thickness of biofilm produced by *E.coli*isolates from fecal samples of sheep

No. of isolate	Thickness of biofilm (mm	No. of isolate	Thickness of biofilm (mm
1	0.8	24	2
2	0.2	25	0.3
3	0.9	26	0.2
4	0.2	27	2
5	0.8	28	0.4
6	0.4	29	0.2
9	0.3	30	0.5
10	0.6	31	0.2
11	0.2	32	0.5
12	0.3	33	0.2
13	0.2	34	0.2
14	0.2	35	0.6
15	0.4	36	0.5
16	0.2	37	0.6
18	0.4	38	0.2
19	0.2	39	0.6
20	0.4	40	0.3
21	0.2	41	0.2
22	0.4	42	0.3
23	0.2	43	0.6

Table.10 Results of sensitivity test of, 16 biofilm producer isolates against 10 of antibiotics

Antibiotic	Sensitive		Intermediate		Resistant	
Bacitracin (10mg)	Number	%	Number	%	Number	%
	0	0	0	0	16	100
Vancomycin(10mg)	0	0	0	0	16	100
Imipenem (10mg)	7	43.75	5	31.5	4	25
Gentamicin (10mg)	14	87.5	0	0	2	12.5
Nalidixic acid(30m)	6	37.5	8	50	2	12.5
Ciprofloxacin(10mg)	11	68.75	0	0	5	31.25
Amoxycillin (30mg)	0	0	0	0	16	100
Chloramphenicol(30mg)	10	62.5	4	25	2	12.5
Ceftriaxone (30mg)	2	12.5	4	25	10	62.5
Tobramycin (10mg)	12	75	2	12.5	2	12.5

Table.11 Results of sensitivity test of, 6 non - biofilm producer isolates against 10 of antibiotics

Antibiotic	Sensitive		Intermediate		Resistant	
Bacitracin (10mg)	Number	%	Number	%	Number	%
	0	0	0	0	6	100
Vancomycin (10mg)	0	0	0	0	6	100
Imipenem (10mg)	6	100	0	0	0	0
Gentamicin (10mg)	6	100	0	0	0	0
Nalidixic acid (30mg)	0	0	3	50	3	50
Ciprofloxacin (10mg)	6	100	0	0	0	0
Amoxycillin (30mg)	0	0	0	0	6	100
Chloramphenicol (30mg)	6	100	0	0	0	0
Ceftriaxone (30mg)	0	0	1	17	5	83
Tobramycin (10mg)	4	67	2	33	0	0

EPS is negatively charged and functions as an ionexchange resin which is capable of binding a large number of the antibiotic molecules that are attempting to reach the embedded biofilm cells (Prakash*et al*., 2003).

Embedded biofilm bacteria are generally not actively engaged in cell division and are smaller in size and less permeable to antibiotics (Thien and O'toole, 2001). Virtually all antimicrobial agents are more effective in killing rapidly-growing cells.
The close contact between bacteria within a biofilm and the matrix may inhibit the penetration of antibiotics through the exopolysaccharid matrix (Jefferson *et al*., 2005).

Biofilm-associated bacteria are often less susceptible to antimicrobial agents and host defenses and,as such, infections involving biofilm may be harder to treat and clear (Yarwood*et al.*, 2004).

References

Alhaj, N.; Mariana, N.S.; Raha, A.R., Ishak, Z. (2007). Prevalence ofantibiotic resistance among *Escherichia coli* from differentsources in Malaysia. *International J. Poult. Sci.* 6(4): 293-297

Al-Ithawy, A. A. (2010). Affected factors on *Pseudomonas aeruginosa* Isolated from deferent environmental sources on biofilm formation, M.Sc. Thesis submitted to the university of Anbar/college of science.

Anderson GG,Goller CC, Justice S, Hultgren SJ, Seed PC(2010) Polysacchride capsule and sialic acid – mediated regulation promote biofilm – like intracellular bacterial communities durin cystitis . Infect Immun 78(3):963-975

Baselga, R.; Albizu, I.; De La Cruz, M.; Del Cacho, E.; Barberan, M. and Amorena, B. (1993). Phase variation of slime production in *Staphylococcus aureus*: implications in colonization and virulence. Inf. Imm. 61, 4857–4862.

Bauer, A.W.; Kirby, W. A.W.; Sherris, J. S. and Turk, M.(1966). Antibiotic susceptibility testing by astandarised single disc method. AM.J. Clin . Patholo ., 45:493 - 496 .

Chapman, P .A; Wright, D. J.; and Siddons, C .A.(1994).A comparison of

immunomegneticsepration and direct culture for the isolation of verocytotoxin –producing *Escherichia coli* form bovine faces . J.Med. Microbiol .40: 424 –427.

Christensen, G .D.; Simpson, W .A.; Bisno, A. L.andBeachey, E .H.(1982). Adherence of slime producing strains of *Staphylococcus epidermidis*to smooth surfaces. Infect Immun; 37:318–26.

Clinical and Laboratory Standards Institute. 2006 PerformanceStandards for Antimicrobial Disk Susceptibility Tests, 9th ed.Approved standard. M2-A9. CLSI, Wayne, Pennsylvania

Donlan, R. M. and Costerton, J. W. (2002). Biofilms: Survival mechanisms of clinically relevant microorganisms. ClinMicrobiol Rev; 15:167-193.

Gorski, N.P. and Palmer, J.N. 2007 Bacterial biofilms in chronic rhinosinusitis (in polish). Mag. Otolaryngologiczny (special edition), February – 2007:3-12

Jefferson, K. K.; Goldmann, D.A.; and Pier, G.B. (2005).Use of confocal Microscopy To Analyze the Rate of Vancomycin Penetration through *Staphylococcus aureus* Biofilms. *Antimicrobial Agents and Chemotherapy*. 49(6): 2467.

Kaper, J .B ., Nataro , J. P . And Mobley, H.L.T. (2004). Pathogenic *Escherichia coli*. Nat. Rev. Microbial 2:123- 140.

Mae□da , A.Y. (2013) . Study comparative protection of biofilm produced by *Staphlococcusaureus* with other it□s antigen , M .Sc .Thesis – College of Veterinary Medicine – Baghdad University .

Mah, T. and O'Toole, G. A. (2001). Mechanisms of biofilm resistance to antimicrobial agents. Trends Microbiol 9:34–39.

Melchior , M.B.; Vaarkamp , H. and Fink – Gremmels , J. 2006 . Biofilms – A role in recurrent mastitis infections . Vet . J. 171:398 -407.

Miles, T.D.; McLaughlin, W. and Brown, P.D. (2006). Antimicrobialresistance of *Escherichia coli* isolates from broiler chickens andhumans. *BMC Vet. Res*., 2: 7.

Nickel, J .C.; Ruseska, I.; Wright,J.B. ;Costerton, J.W.(1985). Tobramycin resistance of *Pseudomonas aeruginosa*cells growing as a biofilm on urinary catheter material. Antimicrob Agents Chemother., 27: 619-624.

Quinn, P.J.; Markey, B.K.; Carter, M.E.; Donnelly, W.J. and Leonard, F.C. (2004). "Veterinary Microbiology and Microbial Diseases Textbook". Printed and bound in Great Britain by International Ltd. Mosby, London, Padstow-Cornwall. : 118-126.

Percival SL , Walker J, Hunter P (2000) Microbiological aspects of biofilms and drinking water . CRC press , New York.

Percival , S.L. ; Derek , C.k.; Christine , A.C.(2011)"Biofilm and veterinary medicine " London : 42

Prakash B, Veeregowda BM, Krishnappa G, (2003). Biofilms: A survival strategy of bacteria. Current Sci. 85: 9, 10.

Sara A. R. (2014). Study the effect of bacterial extracts of filamentous bacteria (*Streptomyces chromofucus*/NS-38) on the bacterium *Staphylococcus aureus*isolated from cases of mastitis in cows. M .Sc .Thesis – College of Veterinary Medicine – Baghdad University .

Schulze, J.; Schiemann, M. and Sonnenborn, U. (2006). 120 years of *E. coli* Its importance in research and medicine. 6th ed. Alfred-Nissle- Gesellschaft, Germany: 11-13.

Tenaillon , O., Skurnik, D., Picard , B., and Denamur , E.(2010). The population

genetics of commensal *Escherichia coli*. Nat Rev Microbial 8: 207- 217

Thien, F.C.M. and O'toole, G.A. (2001). Trends Microbiol., 9: 34-39.

Tunkel, A. R. and Mandell, G. L. (1992). Infecting microorganisms. In: Infective endocarditis. Kaye, D. (ed). New York: Raven Press, pp. 85-97.

Umolu, P.I.; Ohenhen, E.R.; Okwu, I.G. and Ogiehor, I.S. (2006).Multiple Antibiotic resistant index and plasmid of Escherichia coliin beef in Ekpoma. Am. J. Sci. 2 (3):1-4.

Vasudevan, P.; Nair, M.K.M.; Annamalai, T. and Venkitanarayanan, K.S. (2003). Phenotypic and genotypic characterization of bovine mastitis isolates of *Staphylococcus aureus* for biofilm formation. Vet. Microbiol. 92, 179–185

WSPA (World Society for the Protection of Animals) (2006).Resistant bacteria in people and farm animals around the world

Yarwood, J. M.;Bartels, D. J.;Volper, E. M. and Gereenberg, E. P. (2004). Quorum sensing in *Staphylococcus aureus* biofilms. Journal of Bacteriology 186: 1838-1850.

.Zhao,T.; Doyle , M.P.; Shere ,J .(1995) .Prevalence of enterohemorrhagic *Escherichia coli* O157 :H7 in a survey of dairy herds .Appl. Environ. Microbiol .61 :1290 -1293.