RESULTS

And

DISCUSSION

II- RESULTS AND DISCUSSION

II.1. BIOCHEMICAL REACTION

Five different biochemical profiles were identified. Biochemical profile 1 contained 7 bacterial samples (17%). The bacterial enzymatic activity hydrolyzed the sugars Xylose, Maltose, Arabinose, Trehalose, Fructose, Glucose, Lactose, Sorbitol, and Mannitol; and caused carbon utilization for Lysine , Arginine, and Agmatine. These bacterial samples did not produce urease enzyme and did not utilize Citrate.

BIOTYPE:1 (NUMBER OF BACTERIAL SAMPLES: 7)

XYLOSE	+	UREA	-	ORNIHTINE	-	SORBITOL	+
MALTOSE	+	TREHALOSE	+	SUCROSE	-	MANNIITOL	+
ARABINOSE	+	FRUCTOSE	+	INOSITOL	-	ARABITOL	-
MALTONATE	-	LYSINE	+	ESCULIN	-	RAFFINOSE	-
GLUCOSE	+	ARGINNE	+	TDA	-	CELLOBIOSE	-
LACTOSE	+	PYRUVATE	-	CITRATE	-	AGMATINE	+

In biochemical profile 2 there were 12 bacterial samples which represented 30% with enzymatic activity causing hydrolysis of the sugars Maltose, Arabinose, Trehalose, Fructose, Glucose, Lactose, Sucrose, Sorbitol, and Mannitol; and causing carbon utilization for Lysine, Ornithine, and Agmatine.

BIOTYPE:2 (NUMBER OF BACTERIAL SAMPLES: 12)

XYLOSE	-	UREA	-	ORNIHTINE	+	SORBITOL	+
MALTOSE	+	TREHALOSE	+	SUCROSE	+	MANNIITOL	+
ARABINOSE	+	FRUCTOSE	+	INOSITOL	-	ARABITOL	-
MALTONATE	-	LYSINE	+	ESCULIN	-	RAFFINOSE	-
GLUCOSE	+	ARGINNE	-	TDA	-	CELLOBIOSE	-
LACTOSE	+	PYRUVATE	-	CITRATE	-	AGMATINE	+

The enzymatic activity of the bacterial samples present in biochemical profile 3 (9 bacterial samples, 22%) hydrolyzed Xylose, Maltose, Arabinose, Lactose, Glucose, Trehalose, Fructose, Sucrose, Sorbitol, and Mannitol; and utilized the carbon of Lysine, Ornithine, and Agmatine.

BIOTYPE:3 (NUMBER OF BACTERIAL SAMPLES: 9)

XYLOSE	+	UREA	-	ORNIHTINE	+	SORBITOL	+
MALTOSE	+	TREHALOSE	+	SUCROSE	+	MANNIITOL	+
ARABINOSE	+	FRUCTOSE	+	INOSITOL	-	ARABITOL	-
MALTONATE	-	LYSINE	+	ESCULIN	-	RAFFINOSE	-
GLUCOSE	+	ARGINNE	-	TDA	-	CELLOBIOSE	-
LACTOSE	+	PYRUVATE	-	CITRATE	-	AGMATINE	+

There was no enzymatic activity toward Xylose, Malonate, Urea, Arginine, Pyruvate, Inositol, Esculin, Tryptophan, Citrate, Arabitol, Raffinose, and Cellobiose in the bacterial samples included in biochemical profile 4 (7 bacterial samples, 17%).

BIOTYPE:4 (NUMBER OF BACTERIAL SAMPLES: 7)

XYLOSE	-	UREA	-	ORNIHTINE	+	SORBITOL	+
MALTOSE	+	TREHALOSE	+	SUCROSE	+	MANNIITOL	+
ARABINOSE	+	FRUCTOSE	+	INOSITOL	-	ARABITOL	-
MALTONATE	-	LYSINE	+	ESCULIN	-	RAFFINOSE	-
GLUCOST	+	ARGINNE	-	TDA	-	CELLOBIOSE	-
LACTOST	+	PYRUVATE	-	CITRATE	-	AGMATINE	+

The six bacterial samples present in biochemical profile 5 (14%) had an enzymatic activity for Xylose, Maltose, Arabinose, Trehalose, Fructose, Ornithine, Sucrose, Sorbitol, Mannitol, Raffinose, and Agmatine.

BIOTYPE:5 (NUMBER OF BACTERIAL SAMPLES: 6)

XYLOSE	+	UREA	-	ORNIHTINE	+	SORBITOL	+
MALTOSE	+	TREHALOSE	+	SUCROSE	+	MANNIITOL	+
ARABINOSE	+	FRUCTOSE	+	INOSITOL	-	ARABITOL	-
MALTONATE	-	LYSINE	-	ESCULIN	-	RAFFINOSE	+
GLUCOSE	+	ARGINNE	-	TDA	-	CELLOBIOSE	-
LACTOSE	+	PYRUVATE	-	CITRATE	-	AGMATINE	+

All the bacterial samples hydrolyzed the sugars Maltose, Arabinose, Trehalose, Fructose, Sorbitol, and Mannitol; but did not have any enzymatic activity toward Malonate, Urea, Inositol, Esculin, Tryptophan, Citrate, Arabitol, and Cellobiose.

XYLOSE	54%	UREA	0%	ORNIHTINE	84%	SORBITOL	70%
MALTOSE	100%	TREHALOSE	100%	SUCROSE	54%	MANNIITOL	100%
ARABINOSE	100%	FRUCTOSE	100%	INOSITOL	0%	ARABITOL	0%
MALTONATE	0%	LYSINE	86%	ESCULIN	0%	RAFFINOSE	86%
GLUCOSE	100%	ARGINNE	16%	TDA	0%	CELLOBIOSE	0%
LACTOSE	100%	PYRUVATE	0%	CITRATE	0%	AGMATINE	100%

II.2. ANTIBIOTIC SUSCEPTIBILITY TESTING

Four different antibiotic susceptibility patterns were generated among 41 *E. coli* bacterial samples depending upon their susceptibility to 7 different antibiotics.

The twenty bacterial samples in pattern 1 (from bacterial sample 1 to bacterial sample 20) included in pattern 1 their growth was inhibited by nitrofurantoin (MIC ≤ 32 μg/ml), cefotaxime (MIC ≤ 8 μg/ml), gentamicin (MIC≤ 4 μg/ml), and norfloxacin (MIC ≤ 4 μg/ml), and their growth was not inhibited by ampicillin (MIC ≥ 32 μg/ml), piperacillin (MIC ≥ 128 μg/ml), and trimethoprim-sulfamethoxazole (MIC ≥ 4 μg/ml). The minimum inhibitory concentration (MIC) ranged from 2 to 32 μg/ml for nitrofurantoin, from 0.032 to 4 μg/ml for norfloxacin, from 0.032 to 2 μg/ml for cefotaxime, and from 0.25 to 1μg/ml for gentamicin. (Table 1).

Table (1): Antibiotic susceptibility pattern 1

Bacterial sample	F (μg/ml)	SXT (μg/ml)	NOR (μg/ml)	AMP (μg/ml)	PRL (μg/ml)	CTX (μg/ml)	CN (μg/ml)
1	4	R	0.064	R	R	0.19	0.75
2	2	R	0.047	R	R	0.032	0.5
3	6	R	0.094	R	R	0.064	1
4	8	R	0.064	R	R	0.064	1
5	8	R	0.125	R	R	0.094	0.75
6	2	R	0.064	R	R	0.032	1.5
7	24	R	0.5	R	R	0.032	0.5
8	12	R	1	R	R	0.25	1.5
9	16	R	0.25	R	R	0.094	1.5
10	8	R	0.19	R	R	0.19	1.5
11	32	R	4	R	R	2	0.75
12	12	R	0.125	R	R	0.094	1
13	24	R	0.047	R	R	0.047	0.75
14	32	R	1.5	R	R	1.5	1.5
15	32	R	1.5	R	R	0.25	1
16	24	R	0.032	R	R	0.064	0.25
17	12	R	2	R	R	0.38	1.5
18	12	R	0.047	R	R	0.38	0.5
19	16	R	0.25	R	R	0.064	1
20	4	R	0.094	R	R	0.19	0.5

Pattern 2 contained eight bacterial samples (from bacterial sample 21 to bacterial sample 28) which their growth was inhibited by nitrofurantoin (MIC ranged from 0.25 to 32 µg/ml), cefotaxime (MIC ranged from 0.125 to 4 µg/ml), and gentamicin (MIC ranged from 0.38 to 2 µg/ml); and their growth was not inhibited by norfloxacin, Ampicillin, piperacillin, and trimethoprim-sulfamethoxazole (Table 2).

Table (2): Antibiotic susceptibility pattern 2

Bacterial sample	F (µg/ml)	SXT (µg/ml)	NOR (µg/ml)	AMP (µg/ml)	PRL (µg/ml)	CTX (µg/ml)	CN (µg/ml)
21	32	R	R	R	R	0.25	1
22	12	R	R	R	R	0.047	2
23	12	R	R	R	R	0.125	1
24	16	R	R	R	R	1	0.38
25	16	R	R	R	R	0.094	0.25
26	24	R	R	R	R	0.25	0.5
27	32	R	R	R	R	0.125	0.75
28	0.25	R	R	R	R	4	1

Also eight bacterial samples (from bacterial sample 29 to bacterial sample 36) were present in pattern 3 and their growth was inhibited by norfloxacin (MIC ranged from 0.032 to 0.5 µg/ml), cefotaxime (MIC ranged from 0.032 to 1µg/ml), and gentamicin (MIC ranged from 0.38 to 2 µg/ml) (Table 3).

Table (3): Antibiotic susceptibility pattern 3

Bacterial sample	F (µg/ml)	SXT (µg/ml)	NOR (µg/ml)	AMP (µg/ml)	PRL (µg/ml)	CTX (µg/ml)	CN (µg/ml)
29	R	R	0.032	R	R	0.032	0.5
30	R	R	0.19	R	R	0.125	1.5
31	R	R	0.5	R	R	0.032	0.5
32	R	R	0.064	R	R	0.094	0.75
34	R	R	0.25	R	R	0.064	0.5
35	R	R	0.19	R	R	0.5	0.38
35	R	R	0.094	R	R	0.38	1
36	R	R	0.19	R	R	1	2

Finally, the five bacterial samples (from bacterial sample 37 to bacterial sample 41) present in pattern 4 their growth was inhibited by cefotaxime (0.047 - 0.19 µg/ml), and gentamicin (0.5 - 4 µg/ml) (Table 4).

Table (4): Antibiotic susceptibility pattern 4

Bacterial sample	F (µg/ml)	SXT (µg/ml)	NOR (µg/ml)	AMP (µg/ml)	PRL (µg/ml)	CTX (µg/ml)	CN (µg/ml)
37	R	R	R	R	R	0.064	1.5
38	R	R	R	R	R	0.19	1.5
39	R	R	R	R	R	0.19	4
40	R	R	R	R	R	0.064	0.5
41	R	R	R	R	R	0.047	0.5

The antibiotic resistance patterns were pooled, and it was found that 31% of the bacterial samples their growth was not inhibited by nitrofurantoin, 100 % were not inhibited by trimethoprim-sulfamethoxazole, ampicillin, and piperacillin; 31% were not inhibited by norfloxacin, and 0% were not inhibited by cefotaxime and gentamicin (Table 5).

Table (5): Antibiotic types retrieved in this study[a]

Pattern no.	No. of Bacterial sample	Susceptibility of the following antibiotics[b] F (µg/ml)	SXT (µg/ml)	NOR (µg/ml)	AMP (µg/ml)	PRL (µg/ml)	CTX (µg/ml)	CN (µg/ml)
1	20	S (≤ 32)	R(≥4)	S(≤4)	R(≥32)	R (≥128)	S(≤8)	S(≤4)
2	8	S (≤ 32)	R(≥4)	R(≥16)	R(≥32)	R (≥128)	S(≤8)	S(≤4)
3	8	R (≥ 128)	R(≥4)	S(≤4)	R(≥32)	R (≥128)	S(≤8)	S(≤4)
4	5	R (≥128)	R(≥4)	R(≥16)	R(≥32)	R (≥128)	S(≤8)	S(≤4)

[a] Abbreviations: F, nitrofurantoin; SXT, trimethoprim-sulfamethoxazole; NOR, norfloxacin; AMP, ampicillin; PRL, piperacillin; CTX, cefotaxime; CN, gentamicin; S, Susceptible; R, resistant.

[b] Susceptibility testing was performed as discribed by National Committee for Clinical Laboratory Standards (NCCLS) which were used to categorize strains as susceptible or resistant[26].

II.3. RESTRICTION ENZYME DIGESTION

Seven DNA patterns were observed in restriction enzyme digestion. Seven bacterial samples (1, 2, 3, 17, 20, 23, 24) were present in pattern 1 with two bands at 872 and 700 bp. Pattern 2 included 7 bacterial samples (4,5,6,7,8,9,10) with two bands at 1200 and 900 bp. Pattern 3 contained three bacterial samples (11,12,13) with six bands at 1600, 1500, 1400, 1200, 600, and 400 bp. Pattern 4 included five bacterial samples (bacterial samples 14,15,16,17,18,19) with six bands at 1500,1400, 980, 860, 730, and 360 bp. Twelve bacterial samples (26,27,28,29,30,31,32,33,34,36,39,41) were present in pattern 5 with six bands at 1300, 1100, 980, 800, 750, and 360 bp. Four bands, at 1200, 900, 800, and 360 bp, were observed in pattern 6 which included four bacterial samples (21,22,25,40). Only three bacterial samples (35,37,38) were included in pattern 7 with three bands at 1100, 1000, and 700 bp (Figure 5) (Table 6). There is a difference in intensity of bands within the same pattern or between the different patterns.

The seven patterns generated by DNA based method differed by the presence or abscence of one or two single DNA fragment when compared one with another. Sometimes smearing is observed when multipe DNA fragments which differ slightly in length are visible .

Pattern 5 was the common pattern which represented 29%, each of patterns 1 and 2 were 17%, each of patterns 3 and 7 represented 7.5%, pattern 4 represented 12%, and pattern 6 represented 10%.

The band between 800-872 bp was the most common band which generated in 28 bacterial samples, the band between 700-750 bp was generated in 21 bacterial samples, 350-400 bp was generated in 20 bacterial samples, bands at 1300, 100, and 980 bp base pairs were generated in 13 bacterial samples, and the band at 1200 bp was generated in 10 bacterial samples.

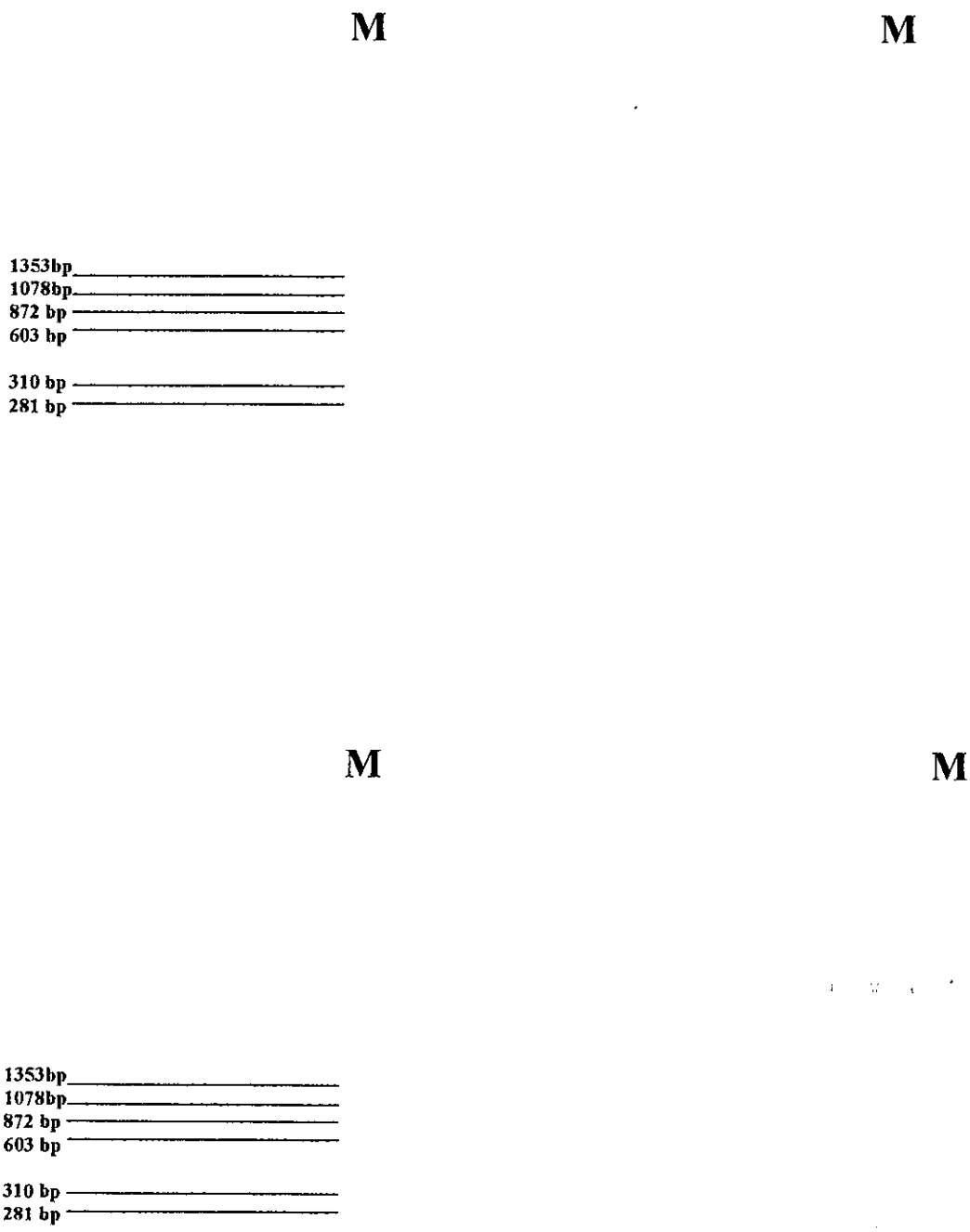

Fig. 5: Representative sample of restriction enzyme digestion of *E. coli* with *Hind III*. M. DNA moleculer weight marker.

Table (6): DNA Patterns

Pattern no.	No. of bacterial samples	DNA fragments observed bp	Bacterial sample numbers
1	7	872,700	1,2,3,17,20,23,24
2	7	1200,900	4,5,6,7,8,9,10
3	3	1600,1500,1400,1200,600,400	11,12,13
4	5	1500,1400,980,860,730,360	14,15,16,18,19
5	12	1300,1100,980,800,750,360	26,27,28,29,30,31,32,33,34,36,39,41
6	4	1200,900,800,350	21,22,25,40
7	3	1100,1000,870	35,37,38

The four patterns resulted from the antibiotic susceptibility testing were divided into seven patterns in the DNA method, i.e. DNA method identified additional heterogeneity among the related strains.

Table (7): Relationship between DNA patterns and antibiotic susceptibility patterns

DNA	Antibiotic susceptibility pattern				Total
Pattern	1	2	3	4	
1	5	2	0	0	7
2	7	0	0	0	7
3	3	0	0	0	3
4	5	0	0	0	5
5	0	3	7	2	12
6	0	3	0	1	4
7	0	0	1	2	3
Total	20	8	8	5	

DNA pattern 1 (7 samples) contained 5 bacterial samples (71%) and 2 bacterial samples (29%) from antibiotic susceptibility pattern 1 and 2 respectively. DNA patterns 2 (7 samples), 3 (3 samples), and 4 (5 samples) included all of their bacterial samples (100%) from antibiotic susceptibility pattern 1. DNA pattern 5 (12 samples) was composed of 7 bacterial samples (58%), 3 bacterial samples (25%), and 2 bacterial samples (17%) from antibiotic susceptibility patterns 3, 2, and 4 respectively. DNA pattern 4 (4 samples) was consisted of 3 bacterial samples (75%) and 1 bacterial sample (25%) from antibiotic susceptibility patterns 2 and 4 respectively. Finally; DNA pattern 7 (3 samples) contained 2 bacterial samples (66%) and 1 bacterial sample (33%) from antibiotic susceptibility patterns 4 and 3 respectively.

Table (8): Percentage of DNA patterns

Antibiotic susceptibility pattern	DNA Pattern						
	1	2	3	4	5	6	7
1	71.4	100	100	100	0	0	0
2	28.6	0	0	0	25	75	0
3	0	0	0	0	58.33	0	33.33
4	0	0	0	0	16.66	25	66.6
Total	100%	100%	100%	100%	100%	100%	100%

Consequently, the antibiotic susceptibility patterns were divided as follows : pattern 1 (20 bacterial sample) was divided into 5 samples (25%), 7 samples (35%), 3 samples (15%) and 5 samples (25%) in DNA patterns 1, 2, 3 and 4 respectively; pattern 2 (8 bacterial samples) was divided into 2 samples (25%), 3 samples (37.5 %), and 3 bacterial samples (37.5 %) in DNA patterns 1, 5, and 6 respectively; pattern 3 (8 bacterial samples) was divided into 7 samples (87.5%), and 1 bacterial sample (12.5%) in DNA patterns 5 and 7 respectively; and pattern 4 (5 bacterial samples) was divided into 2 samples (40%), 1 bacterial sample (20%), and 2 samples (40%) in DNA patterns 5, 6, and 7 respectively.

The rate of heterogeneity of antibiotic susceptibility patterns were :

$$\text{Pattern 1} = \frac{4\ (\text{DNA patterns})}{20\ (\text{samples})} \times 100 = 20\%$$

$$\text{Pattern 2} = \frac{3\ (\text{DNA patterns})}{8\ (\text{samples})} \times 100 = 37.5\%$$

$$\text{Pattern 3} = \frac{2\ (\text{DNA patterns})}{8\ (\text{samples})} \times 100 = 25\%$$

$$\text{Pattern 4} = \frac{3\ (\text{DNA patterns})}{5\ (\text{samples})} \times 100 = 60\%$$

So, pattern 4 had the highest heterogeneity rate (60%), while pattern 1 had the lowest heterogeneity rate (20%).

Table (9): Percentage of antibiotic susceptibility patterns

DNA Pattern	Antibiotic susceptibility pattern			
	1	2	3	4
1	25	25	0	0
2	35	0	0	0
3	15	0	0	0
4	25	0	0	0
5	0	37.5	87.5	40
6	0	37.5	0	20
7	0	0	12.5	40
Total	100%	100%	100%	100%

A new technique was used to identify the bacterial samples. This technique based upon the reaction achieved with 24 biochemical tests predosed and dried into a plate. The tests included classical biochemical media reformulated to allow fluorometric, reading, along with certain new fluorescent tests.

In sugar fermentation tests production of acid resulting from metabolism of the sugar[27] causes a reduction in the fluorescence of the pH sensitive fluorophore indicating a positive result.

The products of metabolizing of Ornithine, Lysine, Arginine, Malonate, Pyruvate, Citrate and Agmatine result in an increase in the pH of the medium which is detected by a fluorescent pH indicator[26,29,30,31].

Urea is combined with a fluorescent indicator which is non-fluorescent at low pH. When Urea is hydrolyzed by urease, Ammonia is produced causing the pH of the well to rise[32]. this causes the indicator to fluoresce.

$$H_2N-CO-NH_2+2H_2O \xrightarrow{\text{Urease}} CO_2+H_2O+2NH_3 \Leftrightarrow (NH4)_2\,CO_3$$

Tryptophan deamination (TDA test) resulted in a coupled reaction in which the formation of a dark color will suppress the fluorescence signal. The lack of a fluorescence signal indicates a positive result[33].

A novel method (E Test) was used for measuring minimum inhibitory concentrations (MICs). E Test based on a combination of the concepts of both dilution and diffusion tests.

The E-test comprise of thin impervious plastic test carrier (5x50 mm) with a continuous exponential gradient of antibiotics immobilized on one side and a reading interpretive scale on the other in µg/ml. The antibiotic gradient can cover a broad concentration range corresponding to approximately 20 antibiotic dilutions. The slopes and concentration ranges can be optimally designed to correspond to clinically elevant minimum inhibitory concentration (MIC) ranges selected for categorization of susceptibility groups (Fig. 6).

The antibiotic gradient is applied onto the surface of an inoculated agar plate. After the required period of incubation whereby bacterial growth becomes visible an inhibition ellipse is seen. The zone edge intersects the length of the graded carrier at a position where a specific concentration of the antibiotic causes an inhibition of the bacterial growth. This value, named the inhibitory concentration (IC), is a direct measure of the susceptibility of the microorganisms for the particular antibiotic (Fig. 7)[34].

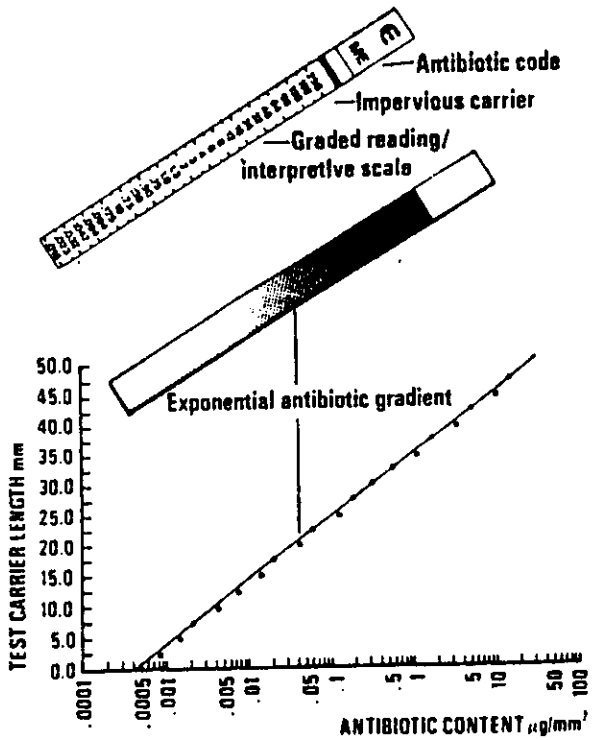

Fig. 6: Configuration of the E Test strip

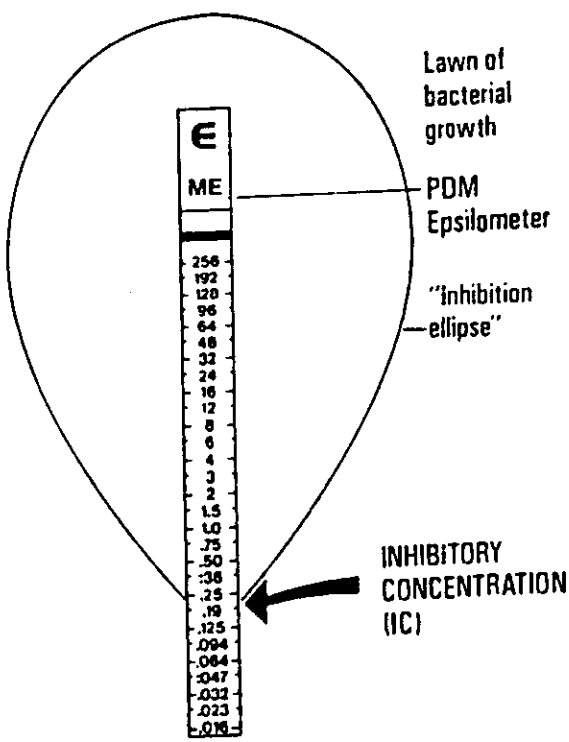

Fig. 7 : Principle of E Test

The antibiotics tested were Nitrofurantoin, Trimethoprim-sulfamethoxazole, Norfloxacin, Ampicillin, Piperacillin, Cefotaxime and Gentamicin.

Nitrofurantoin is a derivative of the furan, 5- membered ring sugar, and possesses a nitro group in the 5-position. Nitrofurantoin inhibits protein synthesis of bacteria. The appear of inhibition preferentially the synthesis of inducible enzymes by blocking the initiation of translation. The resistance to nitrofurantoin is due to decrease of cell permeability[35,36]

The term Sulfonamide is a generic name for derivatives of *p*-Aminobenzyenesulfonamide. In bacteria, Sulfonamides interfere with the synthesis of Folic acid by inhibiting the condensation of *p*-Aminobenzoic acid with 2-Amino-4-hydroxy-6- dihydropteridinylmethyl pyrophosphate to form Dihydropteroic acid and therefore inhibits the bacterial cell wall synthesis[37] .

Sulfonamides can be rendered ineffectively by altered or production of new dihydropteroic synthetase enzyme that has poor affinity for Sulfonamides and preferentially binds p-Aminobenzoic acid[38].

The basic structure of Ampicillin consists of a Thiazolidine ring joined to a β-Lactam ring, to which is attached a side chain. Ampicillin inhibits the cell wall synthesis by interfering with the biosynthesis of peptidoglycan which used in the bacterial cell wall synthesis. Ampicillin is called β-lactam drug because of the importance of the β-lactam ring[39]. An intact ring structure is essential for antibacterial activity; cleavage of the ring by β-lactamase enzymes inactivate the drug. The effect of the enzyme on the β-lactam ring is blocked by modification of the side chain with the addition of large aromatic rings containing bulky methyl or ethyl groups (Piperacillin) [40].

Cefotaxime is also β-lactam drug that act in the same manner as Ampicillin; i.e. inhibits the cross-linking of peptidoglycan. The structure, however, is different: It possesses a β-lactam ring that is fused with a six-membered Dihydrothiazine ring instead of the five-membered Thiazolidine ring. It has a sulfur atom at position 1 of the Dihydrothiazine ring. Substitutions at position 7 or nearly affect the stability against β-lactamases.

Resistance Ampicillin and Cefotaxime can also be due to changes in the penicillin - binding proteins in the bacterial cell membrane. There is another form of resistance, called tolerance, in which growth of the organism is inhibited by Ampicillin or Cefotaxime but the organism is not killed. This is attributed to a failure of activation of the autolytic enzymes, murrain hydrolases, which degraded the peptidoglycan[41].

Aminoglycosides (ex. Gentamicin) are named for the amino sugar component of the molecule, which is connected by glycosidic linkage to other sugar derivatives. The important mode of action of aminoglycosides is misreading of messenger RNA (mRNA) and therefore inhibition of protein synthesis in bacteria[42].

Resistance to aminoglycosides occurs by three mechanisms: (1) modification of the drugs by plasmid-incoded phosphorylating, and acetylating enzymes (the most important mechanism); (2) chromosomal mutation, e.g., a mutation in the gene that incodes for the target protein in the 30S subunit of the bacterial ribosome; and (3) decreasing permeability of the bacterium toward the drugs[43].

Quinolones (ex. Norfloxacin) are bactericidal drugs that block-bacterial DNA synthesis by inhibiting DNA gyrase. Resistance to quinolones is due to the permeability to chromosomal mutations that modify the bacterial DNA gyrase.

Resistance can also be caused by changes in the bacterial outer-membrane proteins that result in reduced uptake of the drug into bacteria[44].

All bacterial samples in the antibiotic susceptibility testing were inhibited by Cefotaxime and Gentamicin, but their growth was not inhibited by Ampicillin, Piperacillin, and Trimethoprim-Sulfamethoxazole.

The minimum inhibitory concentration (MIC) of Nitrofurantoin was ranged from 0.25 µg/ml to ≥ 128 µg/ml, from 0.032 to ≥ 16 µg/ml for Norfloxacin, from 0.032 to 4 µg/ml for Cefotaxime, and from 0.032 to 2 µg/ml for Gentamicin which was the lowest MIC.

Studies of epidemiological markers are important in an attempt to trace the source of contamination or to prevent patient to patient dissemination of strains[45].

Investigation of the epidemiology of *E.coli* infections has become of greater interest and importance because of the relative paucity of antibiotics active against *E.coli* , recent development of multiple antibiotic resistance, and need for bactericidal activity (hence combination of antibiotics) to achieve successful outcomes for some infections. Furthermore, typing of *E.coli* strains is necessary for proper epidemiological investigations of sources and modes of spread of strains in hospital and to design appropriate control measure[46].

The performance of epidemiological typing systems can be evaluated by using several criteria, including typeability, reproducibility, in vivo stability, discriminatory power, and typing system concordance. In addition, typing should meet convenience criteria, like rapidity, accessibility, flexibility and case of use[47,48].

Numerous methods have been proposed to type *E.coli* for epidemiological purposes: Antibiogram, biotyping, serotyping, phage typing, and bacteriocin; the molecular typing methods have been used more recently and are referred in differentiating strains for epidemiological studies[45], plasmid analysis, DNA restriction enzyme analysis[49,50], ribotyping[51,52], PCR[53] and pulsed field gel electrophoresis (PFGE). Phage typing is laborious, of limited utility and available through only a few reference laboratories[54,55]. Antibiograms often suffer from insufficient reproducibility, limited discriminatory power, time-consuming, or poor specimen typeability[56]. Biotyping, serotyping, and bacteriocin typing can not discriminate between strains, or they are lengthily and labor-intensive.

Ribotyping is slower, more labor-intensive and involves working with probes [45]. PFGE requires specialized equipment, and it is a rather time-consuming and labor-intensive method[46]. Whole-plasmid analysis in combination with restriction enzyme digestion of purified plasmid DNA has continued to be a very useful tool in many investigation, but it has many drawbacks such as difficulty in extracting plasmids from bacterial samples, recombination of plasmids from strain resulting inconsistent reproducibility, rearrangements and/or deletions of DNA sequence from plasmids are common events, circular forms of plasmid DNA and chromosomal-DNA contamination complicate interpretation of DNA fragments and the great disadvantage of this method is the instability of the plasmid profiles caused by the acquisition or loss of the plasmids[54,57,58].

In contrast, PCR-based methods use widely available equipment, technically simple and are rapid to perform[46]. Restriction endonuclease analysis of chromosomal DNA has been shown to be useful for the identification of some bacteria[59,60]. Use of an rRNA probe to highlight restriction fragment length polymorphism yielded hybridization banding patterns that allow simpler comparisons of bacterial samples[61].

The characterization of *E. coli* strains using restriction enzyme digestion has shown that this method can readily distinguish genetically distant strains and can also disclose variation among phenotypically related strains[62,63,64].

The present data indicate that restriction enzyme digestion should serve as a first screen for *E. coli* typing because of the simplicity, relatively low cost, and high speed of this technique. This would enable clinical microbiologists to type most of the nosocomial epidemics[62].

Restriction enzyme digestion presented here is relatively rapid and very reproducible reliable method that can be used to type a broad array of bacterial species. It provides same-day results and fulfills adequately the performance criteria proposed recently by several authors