

IN VITRO COMPARATIVE STUDY OF THE EFFICACY OF DIFFERENT BRANDS OF AMOXYCILLIN

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SUMMARY

This study was designed to establish the efficacy of various brands of Amoxicillin, purchased from the local markets of Peshawar. A total of fifteen brands of Amoxicillin, and the raw material (Amoxicillin) were included in the study. Standard Amoxicillin obtained from Oxoid company, London, was used as a control. Discs of Amoxicillin were prepared according to standard procedure and were tested on microorganisms isolated from different specimens in the Microbiology Laboratory of Khyber Medical College, Peshawar. The brands producing a zone of inhibition of ≥ 18 mm were considered effective. The evaluation of data shows that none of the brands of Amoxicillin matched the inhibitory effect of the standard. Some of the products consistently did not produce any inhibitory effect on any of the organisms tested, while others did produce inhibition zone on some of the isolates, but not on others. Even the pure powder of Amoxicillin (the raw material), which was being used by the manufacturers for producing the finished drugs, could not match with standard discs. No difference of efficacy was found in the products of the multinational or the local companies.

INTRODUCTION

The lives of individual patients are put at risk by substandard pharmaceuticals. The major problems arise when substandard antibiotics are prescribed. They not only lead to the development of resistant strains of bacteria but also to treatment failures.¹

There are countries where it has been claimed that over half the drugs in circulation are fake. It is, of course, hard to compile accurate figures for the drug supply of an entire country, but some studies have been conducted on small scale. One such study conducted in Africa shows that of 26 analyzed samples of chloramphenicol 16 were below standard.¹ In another study conducted in Bangladesh almost similar results were obtained, when samples of Ampicillin and trimethoprim/sulphamethoxazole were analyzed.²

Amoxicillin is a semisynthetic antibiotic with a broad spectrum of bactericidal activity against many gram positive and gram negative microorganisms. It is available in oral as well as in parenteral forms. It is rapidly absorbed after oral administration, and diffuses readily into most of the body tissues and fluids with the exception of cerebrospinal fluid. Most of it is excreted unchanged in the urine.³

Amoxicillin is bactericidal against susceptible organisms, during the stage of active multiplication. It acts through the inhibition of biosynthesis of cell wall mucopeptide. In-vitro studies have demonstrated the susceptibility of gram-positive bacteria, i.e alpha and beta hemolytic streptococci, *Diplococcus pneumoniae*, non-penicillinase producing staphylococci and *Streptococcus faecalis*. It is active in vitro

against many strains of *Haemophilus influenzae*, *Neisseria gonorrhoea*, *Escherichia coli* and *Proteus mirabilis*. It is not effective against penicillinase producing bacteria, particularly resistant staphylococci. All strains of *Pseudomonas* and most of the strains of *Klebsiella* and *Enterobacter* are resistant.³⁻⁴

Amoxycillin, like other penicillins, is among the most widely used and least toxic antibiotics available.⁵ The wide spread use of Amoxycillin (because of its broad spectrum antibacterial activity, low cost of treatment and less incidence of adverse effects) prompted us to select Amoxycillin for this study.

MATERIAL AND METHODS

Sixteen brands of Amoxycillin trihydrate acquired from the local market were tested for their efficacy on different bacterial isolates which were isolated in the Microbiology Laboratory of Khyber Medical College Peshawar. The raw material produced in the country which is then used by different manufacturers was also tested. Discs containing 20 mcg of Amoxycillin were prepared from all these samples.⁶

The bottles containing the discs prepared from all the sixteen brands, the raw material and the standard Amoxycillin were designated by different English alphabets at random to avoid any bias or favour.

Following organisms were tested for their susceptibility to these amoxycillins

<i>Escherichia coli</i>	eight isolates
<i>Proteus vulgaris</i>	five isolates
<i>Salmonella typhi</i>	one isolate
<i>Staphylococcus aureus</i>	seven isolates
Beta-haemolytic streptococcus	one isolate
<i>Proteus mirabilis</i>	two isolates
<i>Staphylococcus epidermidis</i>	one isolate
<i>Pseudomonas aeruginosa</i>	one isolate

The organisms were spread on nutrient agar plates and discs of Amoxycillin were applied. Discs of standard Amoxycillin obtained from Oxoid Company London were also applied for comparison. The plates were incubated at 37 °C for 18 hours, and were then examined for the presence or absence of any zone of inhibition around these discs.⁷

These zones and their sizes of inhibition around various discs of different brands of Amoxycillin were compared with the standard discs.⁸

Quantitative methods that require measurement of zone diameters give the most precise estimates of antibiotic susceptibility. With this procedure, a report of "susceptible" indicates that the infecting organism is likely to respond to therapy. A report of "resistant" indicates that the infecting organism is not likely to respond to therapy. A report of "intermediately susceptible" suggests that the organism would be susceptible if high dosage is used, or if the infection is confined to tissues and fluids (e.g. urine) in which high antibiotic levels are attained.⁹⁻¹⁰

RESULTS

All the discs of Amoxycillin prepared, were tested on isolates of *Escherichia coli*, *Proteus vulgaris*, *Salmonella typhi*, *Staphylococcus aureus*, Beta-haemolytic streptococcus, *Proteus mirabilis*, *Staphylococcus epidermidis* and *Pseudomonas aeruginosa*. Results thus obtained are shown in the table-I. The salient features of the findings are produced as follows.

i. *Escherichia coli* (eight isolates)

E, K, O, Q and R were effective (≥ 18 mm) in one; L and P in two and N in three.

A, G and N were intermediately effective (14-17mm) in two; K in five; L in four; O, Q and R in one and P in three.

TABLE 1: EFFECTS OF DIFFERENT BRANDS OF AMOXYCILLIN ON BACTERIAL ISOLATES

Organisms	EFFECT OF DIFFERENT BRANDS OF AMOXYCILLIN (Zone of inhibition in millimeters)																	
	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R
Escherichia coli	—	—	—	—	—	—	—	—	—	24	21	22	14	22	20	20	20	22
	8	—	—	—	11	12	—	10	11	18	11	13	10	16	9	12	14	9
	11	—	—	—	11	11	8	—	8	20	17	16	8	16	12	15	10	—
	15	13	—	10	18	—	14	11	10	22	17	19	10	19	16	18	12	15
Proteus vulgaris	6	6	—	—	11	—	—	—	—	18	17	17	9	18	11	9	9	11
	17	7	—	—	10	6	15	10	11	22	15	15	6	9	10	17	8	9
	—	8	—	—	11	8	8	10	7	18	17	16	9	12	9	15	8	9
	—	—	—	—	—	—	—	—	—	18	13	8	—	8	8	8	—	—
Salmonella Typhi	—	—	—	—	—	—	—	—	—	24	—	—	—	—	—	—	—	—
	15	6	—	—	20	6	7	9	9	24	22	18	10	20	19	20	14	15
	—	—	—	—	—	—	7	—	8	18	—	—	—	10	—	—	—	—
	—	—	—	—	14	—	—	—	—	25	19	16	7	10	—	20	7	—
Salmonella Typhi	10	—	—	—	9	—	—	—	8	21	16	16	—	9	10	15	12	11
	10	—	—	—	17	10	—	—	7	24	16	16	1	12	15	11	15	17

Organisms	EFFECT OF DIFFERENT BRANDS OF AMOXYCILLIN (Zone of inhibition in millimeters)																	
	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R
Staphylococcus aureus	—	—	—	—	—	8	—	8	8	20	—	8	8	—	—	—	12	—
	7	—	—	—	—	9	8	9	—	20	9	—	7	18	10	17	17	9
	17	7	—	—	20	6	15	11	10	22	15	15	6	9	10	17	8	9
	—	—	—	—	—	—	—	—	—	18	—	—	—	—	13	9	—	—
	—	—	—	—	—	—	—	—	—	25	—	—	—	—	—	—	—	—
	—	—	—	—	—	—	—	—	—	17	—	—	—	—	—	—	—	9
	—	—	—	—	—	—	—	—	—	18	8	—	—	8	8	8	—	13
β -haemolytic streptococcus	21	16	12	15	22	18	20	20	19	30	20	18	15	21	24	24	23	12
Proteus mirabilis	15	13	12	14	18	10	18	18	18	24	20	18	16	21	18	19	20	12
	—	—	—	—	17	—	13	8	—	19	10	8	8	9	10	12	9	8
Staphylococcus epidermidis	—	—	—	—	—	—	—	—	—	21	16	—	—	—	8	—	—	—
Pseudomonas aeruginosa	—	—	—	—	—	9	9	—	—	13	—	9	—	8	8	—	9	—

DIFFERENT BRANDS OF AMOXYCILLIN INCLUDING PURE POWDER AND THE STANDARD ARE DESIGNATED BY THE ENGLISH ALPHABETS

J – STANDARD AMOXYCILLIN

O – RAW MATERIAL AMOXYCILLIN)

RESISTANT \leq 13mm

INTERMEDIATE 14-17mm

SUSCEPTIBLE \geq 18mm

– SIGN SHOWS NO EFFECT

- A, G, O, Q and R were ineffective ($\leq 13\text{mm}$) in six; B, C, D, F, H and I in all; K and L in two; E, M in seven and N, P in three.
- ii. *Proteus vulgaris* (five isolates)
- E, L, N and O were effective ($\geq 18\text{mm}$) in one; K and P in two.
- A, E, K, P and Q were intermediately effective (14–17mm) in one and L in two.
- B, C, D, F, G, H, I, M were ineffective ($\leq 13\text{mm}$) in all; A, E, N, O, Q and R in four and K, L and P in two.
- iii. *Salmonella typhi* (one isolate)
- None was effective ($\geq 18\text{mm}$).
- E, K, L, O, Q and R showed intermediate activity (14–17mm).
- iv. *Staphylococcus aureus* (seven isolates)
- N was effective ($\geq 18\text{mm}$) in one.
- A, G, K, L and Q were intermediately effective (14–17mm) in one and P in two.
- A, G, K, L, N and Q were ineffective ($\leq 13\text{mm}$) in six; B, C, D, E, F, H, I, M, O and R in all; and P in five.
- v. Beta-hemolytic streptococcus (one isolate)
- All were effective ($\geq 18\text{mm}$) except B, C, D, M and R.
- B, D and M were intermediately effective (14–17mm).
- C and R were ineffective ($\leq 13\text{mm}$)
- vi. *Proteus mirabilis* (two isolates)
- E, G, H, I, K, L, N, O, P and Q were effective ($\geq 18\text{mm}$) in one.
- A, D and M were intermediately effective (14–17mm) in one.
- A, D, G, H, I, K, L, M, N, O, P and Q were ineffective ($\leq 13\text{mm}$) in one; B, C, F and R in two.

- vii. *Staphylococcus epidermidis* (one isolate)

None was effective ($\geq 18\text{mm}$)

Only K was intermediately effective (14–17mm).

- viii. *Pseudomonas aeruginosa* (one isolate)

All the brands including the standard were ineffective ($\leq 13\text{mm}$).

J (standard Amoxycillin) was effective against all the bacterial isolates except *Pseudomonas aeruginosa*. Beta-hemolytic streptococcus showed intermediate susceptibility (14–17mm) or susceptibility ($\geq 18\text{mm}$) to the majority of the brands. There were no gross differences between the brands produced by the multinational companies (B, K, N and R) and the local companies (rest of the brands).

DISCUSSION

In many developing countries there has been a trend in marketing of spurious drugs that contain little, if any of active ingredients. Samples of Ampicillin and trimethoprim/sulphamethoxazole collected from different parts of Bangladesh have revealed that they were of substandard quality.² Another internationally organized survey conducted in three countries of equatorial Africa was also directed to antibiotics and antiparasitic preparations which revealed that of 26 analyzed samples of chloramphenicol, 16 were below standard and of 49 samples of trimethoprim/sulphamethoxazole tablets, six provided less than the labelled amount, and a further six contained neither of the active ingredients. The survey also revealed that 8 out of 28 samples of ampicillin contained too little of the active ingredient, and one contained none.¹¹ Similarly, in a survey in China, it has been found that 96 of China's 556 pharmaceutical factories were producing substandard drugs.¹²

The above mentioned findings and observations prompted us to conduct similar

studies on in-vitro comparison of the efficacy of different brands of Amoxycillin. Amoxycillin has been picked up for these studies because of the fact that it is very widely used in this country and that we could collect as many as fifteen different brands prepared by both national and international pharmaceutical agencies.

In the present study the evaluation of data showed that all the bacterial isolates except *Pseudomonas aeruginosa* were susceptible to the standard Amoxycillin discs (all strains of *pseudomonas aeruginosa* are resistant to Amoxycillin.³⁻⁴ Rest of the brands including raw material (Amoxycillin) were very much less effective as compared to the standard. Brands labelled K, L, N and to a lesser extent brands P and Q were comparatively better than the rest of the brands. There was no difference in the efficacy of the brands produced by multinational companies (B, K, N and R), and the brands produced by local companies (rest of the brands).

Our findings, though confined to only one drug, are very much in accordance with the appalling scenario prevailing in many other countries. These findings are also in accordance with the serious situation in Pakistan, where it has been recently alleged that about 70% of the licensed drug manufacturers are making substandard drugs.¹³

Prescription of substandard antimicrobial agents not only results in treatment failures, but also leads to the emergence of resistant pathogenic organisms, a situation that has grown to an alarming extent.¹⁴⁻¹⁵ This is also evident from a 1994 WHO report that the continued use of substandard combinations of antitubercular drugs is the major factor involved in the wide spread emergence of resistant tubercle bacilli.¹ The danger is evident: tuberculosis is fast escaping from effective control.¹⁶ As many as 500 incidents of this type triggered by such drugs have been reported which the

experts believe to be only the tip of a very large iceberg.¹⁷

The disregard for Good Manufacturing Practices (GMP) and ignoring the laid down procedures for quality control are the major factors which are responsible for manufacturing of substandard drugs. The situation can be improved by being increasingly vigilant in the struggle against poor quality, low priced drugs.¹⁷ The national drug policy should develop an efficient national drug quality assurance system to ensure that all drugs marketed comply to a set of standards of safety, efficacy and quality that are agreed upon: such an efficient system can only function if we limit the number of registered drugs.¹⁸ There is an urgent need for adapting very strict measure to control the situation. The termination of drug manufacturing licenses for violation of the GMP is a very mild action.¹³

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