*Rev. Inst. Med. trop. São Paulo*
**12(3):195-197, maio-junho, 1970**

*UDC 616.981.452*

# *"IN VITRO"* SENSITIVITY OF 100 STRAINS OF *PASTEURELLA PESTIS* FROM NORTHEASTERN BRAZIL TO ANTIBIOTICS AND A COMPOUND SULPHONAMIDES

Dalva A. MELLO [1]

## SUMMARY

The Author tested 100 strains of *Pasteurella pestis* isolated in Northeastern Brazil with regard to their sensitivity to six antibiotics and one tri-sulpha compound.

Results showed that all the strains were resistant to the sulpha compound. The sensitivity of the 100 plague bacilli strains to the antibiotics was as follows: chloramphenicol 100%, kanamycin and streptomycin 99%, tetracycline 98%, cephalothin 92%, and penicillin 85%.

## INTRODUCTION

The rational treatment of plague was initiated in 1937 when BUTTLE et al. [3] observed the action of sulphonilamide on *Pasteurella pseudotuberculosis* and *Pasteurella septica* in experimentally infected animals. Since 1940, sulphas have been widely applied in the treatment of plague.

Since that time, antibiotics, with streptomycin as the drug of choice, have supervened the sulphas in the treatment of human plague. Other antibiotics have likewise proven to be effective.

The rational use of therapeutic compounds in infectious diseases demands full knowledge of the *"in vitro"* sensitivity of their etiological agents. On the other hand, the indiscriminate use of antibiotics and sulphas has been responsible for the development of resistant strains of microorganisms, and therefore is one of the most important impediments to the successful treatment of individual cases. In addition, this has created obstacles in the control of several infectious diseases.

Until the present, strains of *P. pestis* isolated in Brazil have never been tested regarding their sensitivity to drugs and antibiotics.

Considering the significance of plague as a public health problem in several parts of the world and particulary its position as one the important endemic diseases in many areas of Brazil, the Author undertook the present study in order to determine the sensitivity of 100 strains of *P. pestis* isolated in Northeastern Region to the sulphas and antibiotics.

## MATERIAL AND METHODS

1) *Antibiotics and Drug* — The antibiotics were used in the sensitivity tests according to the following concentrations: streptomycin 25 mcg, kanamycin 30 mcg, chloramphenicol 30 mcg, cephalothin 30 mcg, tetracycline 30 mcg and penicillin 10 units. The first three were provided by Oxoid laboratory while the others were supplied by B.B.L.

Work carried out in the Department of Microbiology of the School of Medicine, University of São Paulo, Brasil

(1) Biologist, Research Center Aggeu Magalhães and Fellow, Regional Center for Investigation and Teaching of Hygiene and Public Health, Recife, Brazil

TABLE I

Per cent results of the sensitivity of 100 strains of *Pasteurella pestis* to antibiotics and sulphatriad

| Strains | Cephalothin 30 μg | Tetracycline 30 μg | Penicillin 10 unit | Kanamycin 25 μg | Streptomycin 25 μg | Chloramphenicol 30 μg | Sulphatriad 300 μg |
|---|---|---|---|---|---|---|---|
| Sensitive | 92 | 98 | 85 | 99 | 99 | 100 | — |
| Resistant | 8 | 2 | 15 | 1 | 1 | — | 100 |

A tri-sulphonamides compound (sulphatriad) produced by Oxoid laboratory, was used at the concentration of 300 mcg.

Both the sulpha and antibiotics compounds were utilized in form of discs.

2) *Culture Media* — The culture medium used in the test with the sulpha was the Oxoid Lab-Lemco Agar. Five per cent of hemolized horse blood was added to this. Blood Agar Base, from the same laboratory, after the addition of five per cent rabbit defibrinated blood was used in the tests with antibiotics.

3) *Strains Employed* — The one hundred strains of *P. pestis* utilized for the experiments were isolated in the interior of the State of Pernambuco during 1966-1967 from the following sources: 16 from humans, 59 from rodents, and 25 from rodent fleas.

The bacteria used as control in this work were: *Escherichia coli* strain K12 J5, fully sensitive to the sulpha and all the antibiotics utilized, and *Escherichia coli* strain 0111 B4 H12, resistant to these same drugs.

All the bacteria utilized in the present experiments were re-isolated in ordinary agar and tested as pure culture. Following that, the bacteria were suspended in 0.85 per cent saline and standardized (Mac-Farland) to $3{,}10^{8}$ microorganisms/ml. From this suspention, broth dilutions in tubes of 10 ml were made until $10^{-2}$ ($3{,}10^{6}$ microorganisms/ml), 5 ml of which were poored on the surface of the respective culture media in Petri dishes. After few minutes of contact the excess of liquid was withdrawn by aspiration. The distribution of the antibiotics and sulpha discs on the surface of the media were made according to the criteria recommended by W.H.O.[7].

The Petri dishes were then incubated at 36°C and readings made after 24 hours by comparison with control tests[7].

## RESULTS

Table I shows the results obtained in the tests.

The sensitivity of 100 plague bacilli strains tested was as follows: chloramphenicol (100%), kanamycin and streptomycin (99%), tetracycline (98%), cephalothin (92%), penicillin (85%) and sulpha (0%). The inhibition zone varied from 15 to 20 mm in diameter.

As expected, the controls behaved accordingly, i.e., the strain of *E. coli* 0111B4H2 did not show any zone to the drugs tested, while the strain *E. coli* K12J5 always presented an inhibition zone of about 20 mm diameter to both sulpha and antibiotics.

## DISCUSSION

Results obtained in the current paper demonstrated that all the strains of plague bacilli isolated in Northeastern Brazil show a high *"in vitro"* sensitivity to all antibiotics herein tested, and particularly to chloramphenicol.

BRYGOO et al.[1,2], studying strains of *P. pestis* isolated in Madagascar, found similar results with antibiotics, though they have not used sulphas.

Strains of *P. pestis* isolated in Vietnam and studied by MARSHALL et al.[4] during 1965-1966, showed complete sensitivity to streptomycin, chloramphenicol and tetracy-

cline. However, when the above strains of plague bacilli were tested against sulphas, the following results were obtained: 28.1% were resistant to 1 mg and 28.2% to 250 mcg of sulphadiazine, while the resistance observed to sulphatiazole was of 28.2% to 250 mcg and 29.8% to 1 mg.

Special attention is called to the resistance presented by all the Brazilian strains of plague bacilli to the sulpha compound tested in this paper, since sulpha and its derivatives are widely employed in treatment of plague, and their use has been recommended by the W.H.O. Expert Committee on Plague [6].

RESUMO

*Sensibilidade a antibióticos e compostos sulfonamídicos,* "in vitro", *de 100 amostras de* Pasteurella pestis *do Nordeste do Brasil*

A Autora estudou *"in vitro"* o comportamento de 100 amostras de *P. pestis* em relação ao cloranfenicol, cefalotina, kanamicina, penicilina, estreptomicina, tetraciclina e um composto de três sulfonamidas.

Os resultados obtidos mostraram que tôdas as amostras apresentaram resistência ao composto sulfonamídico. Quanto aos antibióticos, as amostras mostraram uma sensibilidade de 85% para a penicilina, 92% para a cefalotina, 98% para a tetraciclina, 99% para a kanamicina e a estreptomicina e 100% para o cloranfenicol.

ACKNOWLEDGMENTS

Special thanks are due to Prof. Carlos da Silva Lacaz, Director of the Department of Microbiology of the Faculty of Medicine (University of São Paulo) for the facilities offered during the Author's probation, and to Prof. Luis R. Trabulsi for the scientific orientation given to the present paper.

Thanks are also due to Dr. Jack Finlay for the English revision of the manuscript.

REFERENCES

1. BRYGOO, E. R. & COURAURIER, J. — Action *"in vitro"* des antibiotiques sur 101 souches malgaches de *Pasteurella pestis*. *Ann. Inst. Pasteur* (Paris) **89**:118-121, 1955.

2. BRYGOO, E. R. & RAJENISON, S. — Activité comparée des antibiotiques *"in vitro"* sur *Pasteurella pestis, Cillopasteurella pseudotuberculosis* et *Pasteurella multocida*. *Arch. Inst. Pasteur* (Madagascar) **30**:75-85, 1962.

3. BUTTLE, G. A. H.; PARISH, H. J.; MCLEOD, M. & STEPHENSON, D. 1937 — *In* POLLITZER, R. — Plague. World Health Organization Monograph Series, no. 22. Geneva, 1954.

4. MARSHALL, J. D.; JOY Jr., R. J. T.; AI, N. V.; QUY, D. V.; STOCKARD, J. L. & GIBSON, F. L. — Plague in Vietnam 1965-1966. *Amer. J. Epidem.* **86**:603-616, 1967.

5. POLLITZER, R. — Plague. World Health Organization Monograph Series no. 22. Geneva, 1954.

6. WORLD HEALTH ORGANIZATION — Expert Committee on Plague. Third Report. Technical Report Series, no. 165. Geneva, 1959.

7. WORLD HEALTH ORGANIZATION — Expert Committee on Antibiotics. "Standardization of Methods for Conducting Sensitivity Test". Technical Report Series no. 210. Geneva, 1961.

Recebido para publicação em 24/4/1969.