Study of Common Micro Organisms in Sputum Sample and Culture Sensitivity in Bilaspur City.

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Abstract- *Sputum is a thick fluid produced by the lungs after infection such as bacteria, fungus. Due to the infection sputum colour could be in different colour and appearance. In this research studied on different types of micro-organisms that are sputum produce in human being at Bilaspur city. Many the microscopically examination of expectorated sputum samples is the most commonly followed method in the Microbiological laboratory for diagnosis of lower respiratory tract infections (LRTIs). While pathogenic bacteria all time survive in the air and they are infected to respiratory tract such as streptococcus species, Klebsiella pneumoniae, Pseudomonas aeruginosa, Moraxella catrrhalis, and Morganella species. Enterobacter species, Lower respiratory tract infection are common in the general population, occurring with increased rate of recurrence in older persons and those with chronic diseases or compromised immune function. In this research 50 sputum samples have taken from different sites. The sample of sputum is inoculated in MacConkey agar and blood agar media. Then after the antibiotic susptibility test is performed and found different zone size.*

Keywords- Sputum culture and sensitivity, Disc diffusion method, Indole test, Citrate agar test, Urease agar test, and Triple sugar iron agar test.

I. INTRODUCTION

Sputum is a thick fluid produced by the lungs after infection such as bacteria, fungus. Due to the infection sputum colour could be in different colour and appearance. In this research studied on different types of micro-organisms that are sputum produce in human being at Bilaspur city. Many bacterial species (normal flora) are present in the sputum sample. While pathogenic bacteria all time survive in the air and they are infected to respiratory tract such as streptococcus, Klebsiella, Pseudomonas, Moraxella etc. Lower respiratory tract infection are common in the general population, occurring with increased rate of recurrence in older persons and those with chronic diseases or compromised immune function. A diagnosis is made by culture of respiratory tract secretions, by isolation of a compatible organism from sputum culture. With regard to bacteriological methods, all sputum samples were examined by microscopy, and samples containing a preponderance of leukocytes and a few squamous epithelial cells were considered acceptable for culture, according to accepted criteria. (Bartlett JG et al., 2000).

- Respiratory Tract Infections

Upper respiratory tract is frequently the site of localized or generalized infections. Infections of the respiratory tract may be divided into four groups:

1. Infections of throat and pharynx
2. Infections of middle ear and sinuses
3. Infections of trachea and bronchi
4. Infections of lungs.

- Infections of the Lung

Pneumonia may be defined as inflammation and consolidation of the lung substance which may be caused by microorganisms and non infective physical and chemical agents.

Specimen: Sputum:

Early morning sample which is most purulent. A properly taken specimen with minimal salivary contamination is to be sent to the laboratory as early as possible. Specimen of blood for culture may be useful in diagnosis of lobar pneumonia (C. Dubey and D.K. Maheshwari 2005).

II. MATERIAL AND METHOD

A. Samples:

In this research four sites of sputum samples for the isolation of organisms in my research work. The total number of sputum samples is 50 from four sites. The name of the sites is

- Site- 1:- Shri Ram Care Hospital Bilaspur (C.G.)
- Site- 2:- Ramani Hospital, Bilaspur (C.G.)
- Site-3:- Mark Hospital, Bilaspur (C.G.)

- Site-4:- Walking patient in SRL Lab Bilaspur (C.G.)

B. Glasswares:

- Test Tubes
- Petri Dishes
- Microscope Slides
- Beakers
- Glass Rod

C. Laboratory Equipments

- Incubator (Manufacturer Date – 2014 , Company Name - Digilab)
- Hot air oven (Manufacturer Date – 2014 , Company Name - Digilab)
- Autoclave (Manufacturer Date – 2014 , Company Name - Digilab)
- Laminar air flow (Manufacturer Date – 2014 , Company Name - Coslab)

D. Culture media:

- MacConkey agar
- Blood agar

E. Biochemical Reagents:

- Indole Reagents
- Citrate agar
- Urease agar
- Triple sugar iron (TSI) agar

Kirby–Bauer antibiotic testing or disc diffusion antibiotic sensitivity testing The agar diffusion test (Kirby–Bauer antibiotic testing, KB testing, or disc diffusion antibiotic sensitivity testing) is a test of the antibiotic sensitivity of bacteria. It uses antibiotic-impregnated wafers to test the extent to which bacteria are affected by those antibiotics. In this test, wafers containing antibiotics are placed on an agar plate where bacteria have been placed, and the plate is left to incubate. If an antibiotic stops the bacteria from growing or kills the bacteria, there will be an area around the wafer where the bacteria have not grown enough to be visible. This is called a zone of inhibition.

The size of this zone depends on how effective the antibiotic is at stopping the growth of the bacterium. A stronger antibiotic will create a larger zone, because a lower concentration of the antibiotic is enough to stop growth.

The bacterium in question is swabbed uniformly across a culture plate. A filter-paper disk, impregnated with the compound to be tested, is then placed on the surface of the agar. The compound diffuses from the filter paper into the agar. The concentration of the compound will be highest next to the disk, and will decrease as distance from the disk increases. If the compound is effective against bacteria at a certain concentration, no colonies will grow where the concentration in the agar is greater than or equal to the effective concentration. This is the zone of inhibition. This along with the rate of antibiotic diffusion is used to estimate the bacteria's sensitivity to that particular antibiotic. In general, larger zones correlate with smaller minimum inhibitory concentration (MIC) of antibiotic for that bacterium. Inhibition produced by the test is compared with that produced by known concentration of a reference compound. This information can be used to choose appropriate antibiotics to combat a particular infection (Mohanty A 2010).

F. Antibiotics:

We are using following antibiotics:

- Ceftriaxone/tazobactam (30/10 mcg)
- Ceftazidime/tazobactam (30/10 mcg)
- Pipracillin/tazobactam (100/10 mcg)
- Cefoperazone/sulbactam (50/50 mcg)
- Amikacin (30 mcg)
- Gentamicin (10 mcg)
- Imipenem (10 mcg)
- Tobramycin (10 mcg)
- Tigecycline (15 mcg)
- Erythromycin (15 mcg)
- Azithromycin (15mcg)
- Chloramphenicol (30 mcg)

Table 1. Sample collection of different site

S.No.	Sample	Total samples	Sites of Samples
1	Sputum	20	Site- 1:- Shri Ram Care Hospital Bilaspur (C.G.)
2	Sputum	10	Site- 2:- Ramani Hospital, Bilaspur (C.G.)
3	Sputum	10	Site-3:- Mark Hospital, Bilaspur (C.G.)
4	Sputum	10	Site-4:- Walking patient in SRL Lab Bilaspur (C.G.)

Table 2. Zone size Interpretative chart for Antibiotics as per CLSI (Based on Results obtained using Mueller Hinton Agar)

S.No.	Antimicrobial Agent	Symbol	Disc Content	Diameter of zone of inhibition in mm		
				Resistant (mm or less)	Intermediate (mm)	Sensitive (mm or more)
1.	Amikacin	AK	30 mcg	14	15-16	17
2.	Azithromycin	AZM	15 mcg	13	14-17	18
3.	Cefoperazone/sulbactam	CFS	50/50 mcg	23	24-25	28
4.	Ceftazidime/tazobactam	CAT	30/10 mcg	17	18-20	21
5.	Ceftriaxone/tazobactam	CIT	30/10 mcg	17	18-20	21
6.	Chloramphenicol	C	30 mcg	12	13-17	18
7.	Erythromycin	E	15 mcg	13	14-22	23
8.	Gentamicin	GEN	10 mcg	12	13-14	15
9.	Imipenem	IPM	10 mcg	19	20-22	23
10.	Pipracillin/tazobactam	PIT	100/10 mcg	17	18-20	21
11.	Tigecycline	TGC	15 mcg	15	16-17	18
12.	Tobramycin	TOB	10 mcg	12	13-14	15

Note: In accordance to performance Standards for Antimicrobial Disc Susceptibility Test, CLSI (Formerly NCCLS).

III. OBSERVATION AND RESULTS

All samples are cultured on MacConkey agar, Blood agar. These plates are incubated at 370C aerobically and after overnight incubation, and then checked for bacterial growth. The organisms are identified by their colony morphology, staining characters, pigment production, motility and other relevant biochemical tests as per standard laboratory methods of identification.

Figure 1. After incubation of MacConkey agar plate

Figure 2. After incubation of Blood agar plate

Table 3. Antibiotic sensitivity zone size of Klebsiella pneumoniae in S1 Sample

S.No.	Name of antibiotics	Zone size	Resistant	Intermediate	Sensitive
1	Amikacin	17	14	15-16	17
2	Azithromycin	14	13	14-17	18
3	Cefoperazone/sulbactam	22	23	24-25	28
4	Ceftazidime/tazobactam	21	17	18-20	21
5	Ceftriaxone/tazobactam	24	17	18-20	21
6	Chloramphenicol	00	12	13-17	18
7	Erythromycin	11	13	14-22	23
8	Gentamicin	15	12	13-14	15
9	Imipenem	24	19	20-22	23
10	Pipracillin/tazobactam	22	17	18-20	21
11	Tigecycline	18	15	16-17	18
12	Tobramycin	14	12	13-14	15

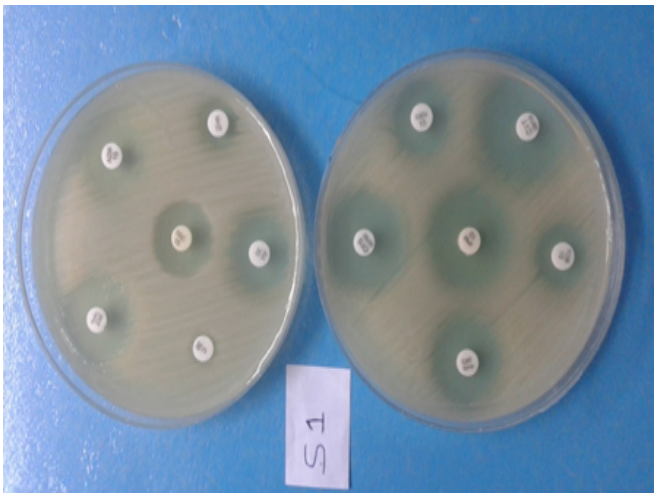

Figure 3. Antibiotic sensitive test of K. pneumonia

Table 4. Antibiotic zone size of K. pneumonia in different sputum samples

S. No.	Name of sputum sample	Name of Antibiotics											
		AK	AZM	CFS	CAT	CIT	C	E	GEN	IPM	PIT	TGC	TOB
1	S1	17	14	22	21	24	0	11	15	24	22	18	14
2	S16	17	8	20	19	22	17	0	15	21	19	17	13
3	S17	16	12	17	18	21	20	0	15	21	18	18	14
4	S20	0	0	0	12	12	14	0	0	0	0	16	0
5	S22	18	16	24	25	25	0	12	24	24	24	23	18
6	S26	16	17	21	21	23	21	9	16	21	19	21	17
7	S32	15	15	22	21	23	13	8	17	21	17	19	15
8	S38	17	11	20	21	22	20	9	15	18	19	21	14
9	S39	16	13	20	21	22	21	0	15	22	18	22	16
10	S42	17	10	22	21	21	21	0	18	17	19	19	19
11	S46	18	15	25	25	27	22	0	17	24	21	20	22
12	S47	21	14	23	22	22	0	0	19	20	21	19	20
13	S50	0	9	12	0	13	21	0	0	18	16	20	0

Note – Red colour box show high antibiotic sensitivity zone size in K.pneumoniae

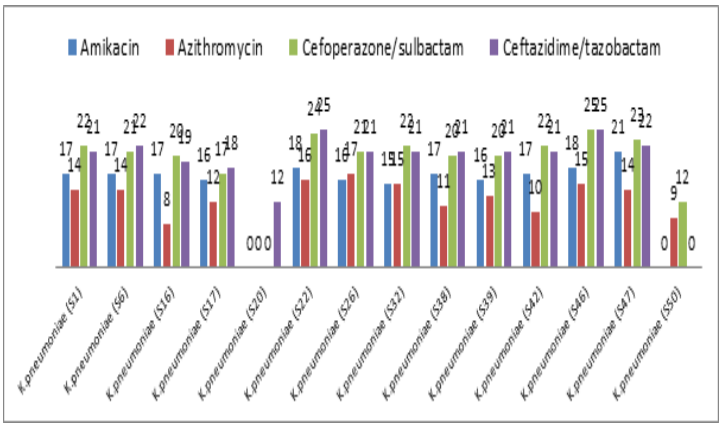

Figure 4. raph No. 1.1 Graphical representation zone size of antibiotic sensitivity test of K.pneumoniae in sputum samples

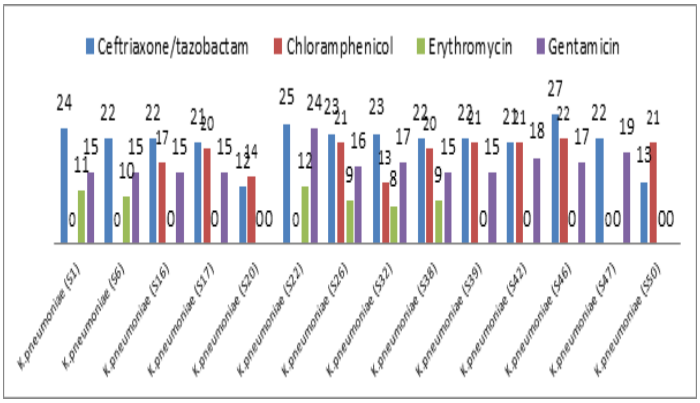

Figure 5. raph No. 1.2 Graphical representation zone size of antibiotic sensitivity test of K.pneumoniae in sputum samples

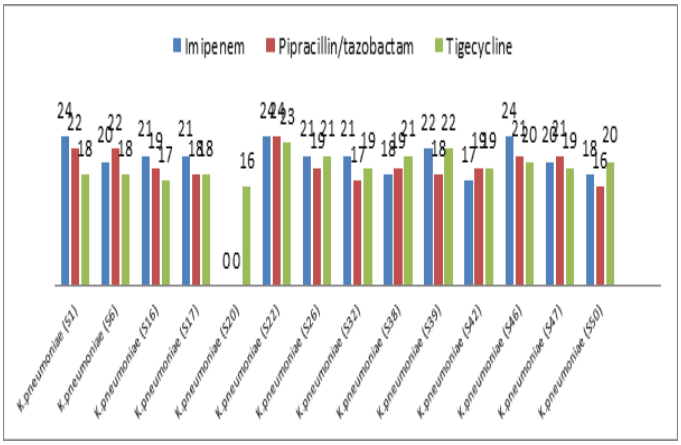

Figure 6. raph No. 1.3 Graphical representation zone size of antibiotic sensitivity test of K.pneumoniae in sputum samples

On my research work we have found following biochemical reactions for the identification of microorganisms which is shows in table form:

Table 5. shows biochemical reactions for the identification of microorganisms

S.No.	Name of microorganisms	Catalase	Coagulase	Indole	Citrate	Urease	TSI	Oxidase
S1	*Klebsiella pneumoniae*	-	-	-	+	+	a/a	-
S2	*Pseudomonas aeruginosa +Enterococcus species*	+	-	-	+	-	k/k	+
S3	*Acinetobacter species*	-	-	-	-	-	k/a	-
	'tazobactam" Point "K.pneumoniae (S26)"		-					
			-	+	-	-	a/a	-
	+Enterococcus species							
S6	*Klebsiella pneumoniae*	-	-	-	+	+	a/a	-
S7	*Pseudomonas aeruginosa*	+	-	-	+	-	k/k	+
S8	*Moraxella catrrhalis*	-	-					+
S9	*Moraxella catrrhalis*	-	-					+
S10	*Moraxella catrrhalis*	-	-					+
S11	*Negative*							
S12	*Negative*							
S13	*Enterobacter species +Enterococcus species*			+	-	-	k/a	-
S14	*Pseudomonas aeruginosa*	+	-	-	+	-	k/k	+
S15	*Negative*							
S16	*Klebsiella pneumoniae*	-	-	-	+	+	a/a	-
S17	*Klebsiella pneumoniae*	-	-	-	+	+	a/a	-
S18	*Enterobacter species*	-	-	+	-	-	k/a	-
S19	*Escherichia coli*							
S20	*Klebsiella pneumoniae*	-	-	-	+	+	a/a	-
S21	*Escherichia coli*	-	-	+	-	-	a/a	-
S22	*Klebsiella pneumoniae*	-	-	-	+	+	a/a	-
S23	*Pseudomonas aeruginosa*	+	-	-	+	-	k/k	+
S24	*Escherichia coli*	-	-	+	-	-	a/a	-
S25	*Escherichia coli*	-	-	+	-	-	a/a	-
S26	*Klebsiella pneumoniae +Enterococcus species*	-	-	-	+	+	a/a	-
S27	*Escherichia coli*	-	-	+	-	-	a/a	-
S28	*Negative*							
S29	*Morganella species*	-	-	-	-	+	k/a	-
S30	*Negative*							
S31	*Escherichia coli*	-	-	+	-	-	a/a	-
S32	*K*	Horizontal (Category) Axis		-	+	+	a/a	-
S33	*Negative*							
S34	*Negative*							
S35	*Pseudomonas aeruginosa*	+	-	-	-	+	k/k	+
S36	*Acinetobacter species*							
S37	*Morganella species*	-	-	-	-	+	k/a	-
S38	*Klebsiella pneumoniae*	-	-	-	+	+	a/a	-
S39	*Klebsiella pneumoniae*	-	-	-	+	+	a/a	-
S40	*Pseudomonas aeruginosa*	+	-	-	+	-	k/k	+
S41	*Moraxella catrrhalis*	-	-	-	-	-		+
S42	*Klebsiella pneumoniae*	-	-	-	+	+	a/a	-
S43	*Pseudomonas aeruginosa*	+	-	-	+	-	k/k	+
S44	*Escherichia coli*	-	-	+	-	-	a/a	-
S45	*Negative*							
S46	*Klebsiella pneumoniae*	-	-	-	+	+	a/a	-
S47	*Klebsiella pneumoniae*	-	-	-	+	+	a/a	-
S48	*Negative*							
S49	*Negative*							
S50	*Klebsiella pneumoniae*	-	-	-	+	+	a/a	-

IV. DISCUSSION

In this research the K.pneumoniae micro-organisms show maximum zone size in all sputum samples such as antibiotic of Amikacin show 21 mm zone size in S31 sputum sample, the antibiotic of Azithromycin show 17 mm zone size in S26 sputum sample, the antibiotic of Cefoperazone/sulbactam show 25 mm zone size in S46 sputum sample, the antibiotic of Ceftazidime/tazobactam show 25 mm zone size in S22 and S46 sputum sample, the antibiotic of Ceftriaxone/tazobactam show 27 mm zone size in S46 sputum sample, the antibiotic of Chloramphenicol show 22 mm zone size in S46 sputum sample, the antibiotic of Erythromycin show 12 mm zone size in S22 sputum sample, the antibiotic of Gentamicin show 24 mm zone size in S22 sputum sample, the antibiotic of Imipenem show 24 mm zone size in S1, S22 and S46 sputum sample, the antibiotic of

Pipracillin/tazobactam show 24 mm zone size in S22, the antibiotic of Tigecycline show 23 mm zone size in S22 sputum sample, the antibiotic of Tobramycin show 22 mm zone size in S46 sputum sample.

V. CONCLUSION

In this research the respiratory specimen (sputum) is sensitive to broad spectrum antibiotics. Hospital acquired patient are mostly resistant to certain small and broad spectrum antibiotics which shows gram negative bacteria. When we used antibiotic culture and sensitivity method and found sensitive antibiotic which inhibit the growth of gram positive and gram negative bacteria. Mostly sensitive antibiotic are carbapenem and cephalosporin group of antibiotic in this research. In bilaspur city mostly gram negative bacteria are responsible for causing pulmonary disease in human beings. So we can help of such kind of patient that are infected with Tuberculosis and other respiratory disease by the process of antibiotic sensitivity test or disc diffusion method.

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