89196_Auto_Edited.docx

WORD COUNT 3878
TIME SUBMITTED 05-JAN-2024 11:44AM
PAPER ID 105935016

Name of Journal: *World Journal of Methodology*
Manuscript NO: 89196
Manuscript Type: REVIEW

Update on the gut microbiome in health and diseases

Maurizio Salvadori, Giuseppina Rosso

Abstract

Abstract: The Human Microbiome Project, Earth Microbiome Project and Next-gen sequencing have advanced novel genome association, host genetic linkages and pathogen identification. Microbiome is the sum of the microbes, their genetic information and their ecological niche. This study will describe how millions of bacteria in the gut affect the human body in health and disease. The gut microbiome changes in relation with age, with an increase of Bacteroidetes and Firmicutes. Host and Environmental factors affecting the gut microbiome are diet, drugs, age, smoking, exercise and host genetics. In addition, changes in the gut microbiome may affect local gut immune system and systemic immune system. In this study, we discuss how microbiome may affect the metabolism of healthy subjects or may affect the pathogenesis of metabolism generating metabolic diseases.

INTRODUCTION

Name of journal: World Journal of Methodology

Manuscript type: Review

Update on the gut microbiome in health and diseases

Salvadori M *et al* Update on the gut microbiome

Maurizio Salvadori, Giuseppina Rosso

Maurizio Salvadori Renal Unit Dept of Transpantation Careggi University Hospital viale Pieraccini 18, 50139 FLORENCE ITALY

Giuseppina Rosso Division of Nephrology San Giovanni di Dio Hospital Florence 1, *via* di Torregalli 1 50143 FLORENCE ITALY

Author contribution: Maurizio Salvadori and Giuseppina Rosso equally contributed to the manuscript. Both authors wrote, controlled and approved the manuscript.

Conflict of interest: Maurizio Salvadori and Giuseppina Rosso do not have any conflict of interest in relation to the manuscript, as in the attached form

Correspondence to: Maurizio Salvadori MD Renal Unit Careggi University Hospital viale Pieraccini 18 50139 Florence Italy

e-mail address: maurizio.salvadori1@gmail.com

tel 0039 055 597151

fax 0039 055 597151

Title: Update on the gut microbiome in health and disease

Abstract: Three relevant projects, the Human Microbiome Project, the Earth Microbiome Project and the Next-generation sequencing Project have advanced novel genome association, host genetic linkages and pathogen identification. Microbiome is the sum of the microbes, their genetic information and their ecological niche. This study will describe how more than 10^{14} of bacteria in the gut affect the human body in health and disease. The gut microbiome changes in relation with age, with an increase of Bacteroidetes and Firmicutes. Host and environmental factors affecting the gut microbiome are diet, drugs, smoking, exercise and host genetics. In addition, changes in

the gut microbiome may affect local gut immune system and systemic immune system. In this study, we discuss how gut microbiome may affect the metabolism of healthy subjects or may affect the pathogenesis of metabolism generating metabolic diseases. In particular, several axis will be analyzed as gut-brain axis, gut-lung axis and gut-heart axis. Attention will be given to the relationship between gut dysbiosis and intestinal diseases as irritable or inflammatory bowel diseases. Finally, the association between gut microbiome and metabolic diseases as obesity, diabetes and cardiovascular diseases will be described.

In the last chapter, both possible treatments and future treatments will be described. Finally, to date techniques and most promising future techniques will be analyzed.

Introduction

More than 150,000 papers with "Microbiome" in title or abstract have been published since the term was introduced in 2001.

Early-stage reports were cross-sectional studies of the microbiota at different body sites, associations with disease markers and diseases themselves. More recently, three relevant projects, the Human Microbiome Project (1), the Earth Microbiome Project (2) and Next-generating sequencing (3), have advanced novel genome associations, host genetic linkages, and pathogen identification.

Current studies principally focus on the functional or mechanistic aspects of differences in microbial composition.

In particular, a study from Gao *et al* (4) identified that the more relevant studies covers six important aspects of microbiome researches. They include best practices for analyzing microbiomes, the regulation of gut microbiomes on the human immune system, how microbiomes affect human immune sensing, the role of microbiomes in gut-brain interaction, the application of microbiomes in maternal and newborn health and the study of nutria-microbiome epidemiology.

Before discussing the gut microbiome, its function and its relationship with health and disease, exact definitions are needed for a correct understanding.

Definitions

Frequently, the terms Microbiota and Microbiome are mutually used, but they have a different significance.

Microbiota are defined as the microorganisms (bacteria, archaea, viruses, fungi) that live in the human body in healthy conditions. All these microorganisms make up the microbiome.

The microbiome is the sum of microbes, their genetic information and their ecological niche.

The metagenome is the totality of genes from the genomics of a mixed microbial population that provides information about genetic potential.

Indigenous microbiota are the resident microbiota resident in the healthy subjects. Dysbiosis is the change in the indigenous microbiota composition causing disease. Pathobionts are the microbiota causing disease.

Gut microbiome

The microbiome is spread across different organs and tissues of the human body, but the most important and the best studied is the gut microbiome.

A total of 10^{14} bacteria already represent the gut microbiome, and 10^{11} bacteria each day flow from the pharynx to the stomach.

Changes in the gut microbiome are associated with diseases, but frequently, it is not known if this is a cause or an effect.

Under normal conditions, the formation of the adult microbiome occurs over the first three years of life and is affected by life events such as weaning, starting solid food, and primarily cessation of breastfeeding. At birth, the most common bacteria are aerobic bacteria such as Enterococcus and Staphylococcus. Later, anaerobes prevail, with a prevalence of Firmicutes and Bacteroidetes (5). Several studies (6-8) have documented the distribution of the normal gut flora in the different parts of the intestine in adults as shown in Table 1.

Factors influencing the gut microbiome

The Human Microbiome Project (1) data suggest that the unperturbed microbiome is stable over short periods and that there is a degree of resilience of the microbiome due to several factors.

In addition to age, several factors affect the composition of the gut microbiome, such as diet, host genetics, exercise, smoking and drugs (9).

A vegetarian and rich fiber diet has a beneficial effect on the gut microbiome, favoring an increase in Firmicutes and Bacteroidetes (10, 11).

Several genes associated with innate immunity influence the microbiome (12). A recent study from Chen *et al* (13) documented the influence of host genetics on the gut microbiome.

Exercise is associated with a beneficial effect on gut microbiome composition, as documented by Hughes *et al* (14) and Allen *et al* (15). It has also been documented that athletes have a reduced rate of inflammatory markers (16) and exercise has been proposed to reduce dysbiosis (17).

No smoker subjects have shown an increase in the fecal microbiota of Firmicutes and Actinobacteria (18). The same author in a different study found differences in the oral gut microbiota in smokers with respect to no smokers (19).

Several drugs have a powerful effect on microbiome composition.

Antibiotics may damage the microbiome in two different ways. On the one hand, by destroying beneficial microbes, antibiotics may cause dysbiosis (20). On the other hand, destroying beneficial microbes blocks the mechanism by which they inhibit pathogens (21). These effects on the gut microbiome are related to the type of antibiotics and the treatment duration. Worse effects on the microbiome have been described with the use of clindamycin (22), clarithromycin, ciprofloxacin (23) and vancomycin (24). All these antibiotics cause a reduction in Bacteroides variety and Ruminococcus.

Concerning non antibiotic drugs, there is a complex bidirectional interaction between their use and the gut microbiome (25)

On the one hand, these commonly used drugs alter the gut microbiome composition and function; on the other hand, gut microbes can contribute to drug efficacy and safety

by enzymatically transforming drug structure and altering drug bioavailability, bioactivity or toxicity. Knowing these interactions enables interventions to modulate the gut microbiome and optimize treatment efficacy (25).

According to the Belgium Flemish cohort (26) and the TwinsUK cohort (27), the most common no antibiotic drugs associated with microbiome modification and dysbiosis are proton pump inhibitors (PPIs), statins, laxatives, metformin and ACE inhibitors.

PPIs, by changing microbiome composition may favor the colonization of pathogens such as *Clostridium difficile* and Salmonella (28, 29).

Metformin, used to treat type 2 diabetes, increases *Escherichia Coli* and reduces Intestinibacter, favoring dysbiosis and causing gastrointestinal side effects (30, 31).

Studies conducted in the UK, the Netherlands and Belgium have documented modifications in microbiome composition after the prolonged assumption of laxatives, statins and antidepressants (26, 27, 32).

Functions of the microbiome

Metabolic function

The gut microbiota has the capacity to metabolize dietary fibers not metabolized by digestive enzymes (33). In this way, the microbiome provides additional energy by metabolizing large polysaccharides and alcohols. The MEROPS database showed that the microbiome produces proteases that are able to metabolize different substances in the large intestine (34, 35). Additionally, beneficial effects produced by the microbiome are related to the production of several vitamins (36, 37). In a study from Afzaal *et al.* are shown the principal metabolites produced by gut microbiota in normal conditions and their functions [38] (table 2) [39-49].

Structural function

Under normal conditions, the microbiome contributes to maintaining the integrity of the gut epithelium. In this condition, cytokines present in the gut lumen do not pass through the gut epithelium. This function may be altered by pathogens such as

Escherichia Coli and *Clostridium difficile*. The dysbiosis produced by these bacteria facilitates the back diffusion of cytokines (50,51).

Protective function

The intestinal surface represents an important barrier, and the microbiome contributes to its stability (52). The production of short-chain fatty acids (SCFAs) by the microbiome provides further energy for the epithelium and strengthens this barrier (53,54).

Table 3 shows examples of gut microbiome-derived metabolites and their beneficial effects in healthy conditions. In order of the metabolites, the pathway involved, the microbial agent responsible and the health benefits produced, this information is shown in Table 3 (55-70).

Relationship between the gut microbiome and immune system

The gut microbiome has important effects on the local and general immune systems.

The local gut microbiome drives the maturation of gut-associated lymphoid tissue (GALT) (71) and maintains barrier function by mucus production and antimicrobial peptide production. In addition, GALT has specific influences on inflammatory *vs* no inflammatory cell phenotypes. One-sixth of the cells of the gut epithelium are represented by T lymphocytes. In addition, B-lymphocytes, dendritic cells and plasma cells are present in lymphoid tissue, principally in the colon mucosa or in Payer's patches (72, 73).

Indigenous microbiota or pathobionts may differentiate Th cells into Th1, Th2, Th17 and Treg cells *via* the production of microbiota metabolites. Filamentous bacteria (SFB) induce the growth of Th 17 and Th 1; Clostridia stimulate Treg and the anti-inflammatory cytokine IL-10. Bacteroides fragilis stimulates IL-10 and Tregs. In contrast, excessive stimulation of Th1 and Th2 cells due to a condition of dysbiosis may cause excessive production of proinflammatory cytokines (74).

Intestinal colonization has an important role in the development of tolerance (75).

This fact is relevant in the prevention of immune-mediated diseases.

Indeed, the loss of immune tolerance may cause the development of allergic diseases or autoimmune diseases. Dysbiosis caused by *Escherichia Coli* or Clostridium difficile is associated with eczema or atopic dermatitis.

The production of SCFAs has a double effect. They constitute the main energy substrate for enterocytes and stimulate the maturation and correct function of Tregs (76).

Several axes have been established between the gut microbiome and different organs. In the case of dysbiosis they can generate diseases.

The gut-Brain Axis may generate stress, anxiety, depression, schizophrenia, cognitive decline, autism.

The Gut-Brain Endocrine Axis generates regulatory, metabolic, behavioral and hormonal disorders.

The Gut-Heart Axis generates cardiovascular diseases, atherosclerosis, thrombotic events, and hypertension.

The Gut-Lung Axis generates chronic obstructive pulmonary disease.

The Gut-Liver Axis generates liver inflammation, hepatocellular carcinoma, and non-alcoholic fatty liver.

The Gut-Pancreas Axis generates diabetes, pancreas cell inflammation.

The Gut-Bone Axis generates bone demineralization, osteoporosis.

The Gut-Muscle Axis generates muscle impairment, fragility, sarcopenia.

The Gut- Skin Axis generates acne, psoriasis, atopic dermatitis, wrinkles, and aging.

The Gut-Reproductive Axis generates infertility, ovarian dysfunction, ovarian cancer, postmenopausal osteoporosis.

The Gut-Kidney Axis generates chronic kidney disease, acute kidney injury, inflammation, nephrolithiasis, and nephropathy.

The Gut Bladder Axis generates urinary tract infection, overactive painful bladder.

Some of these axes will be discussed as follows.

<u>The gut microbiome and the brain</u>

Recently, significant studies have documented the existence of a so-called "gut-brain axis". This is a bidirectional communication system between the gut and brain. On the

one hand, the brain may control gastrointestinal functions such as peristalsis, mucus production and the gut immune system (77). On the other hand, the gut microbiome releases SCFAs and other metabolites influencing brain function, whereas several neurotransmitters are involved in the bidirectional communication between the host and the microbiota (78).

The gut microbiome may affect the brain directly by the gut nervous system sending signals to the brain or indirectly by the production of intestinal hormones or transforming diet components into substances such as SCFAs, neurotransmitters such as serotonins, gamma amino butyric acid (GABA), tryptophan and vitamins that influence the blood brain barrier (BBB) and cerebral functions (79).

In healthy conditions, bacteria such as Lactobacillus, Bifidobacterium, Enterococcus and Streptococcus are among the principal producers of neurotransmitters (80, 81). In addition, SCFAs affect the BBB, and several other immune pathways affect behavior, memory and locomotion (82, 83).

It should also be highlighted that the Mediterranean diet rich in vegetables and fibers stimulates the activity and growth of beneficial bacteria for the brain (84).

For a long time, it has been known that more than 20% of patients with gut dysbiosis are affected by sleep disorders and depression. This fact has been confirmed in recent studies (85, 86).

Similarly, recent studies have documented that microbiota composition differs significantly between healthy controls and patients affected by neurovegetative disorders such as multiple sclerosis (MS), Alzheimer's disease (AD) and Parkinson's disease (PD) (87). In MS, a higher abundance of Firmicutes and the absence of Fusobacteria are frequently found (88). The stool microbial profile of AD patients has a decreased number of Firmicutes and Actinobacteria. Firmicutes, such as Ruminococcoceae and Turicibacteriaceae, are less abundant in these patients (89). PD patients have a lower production of SCFAs and fewer gut bacteria, such as Lechnospiraceae and Faecalibacterium prausnitzii producing these substances (90).

In conclusion, several recent studies have documented that a different composition of the gut microbiome may contribute to the development of neurodegenerative disorders causing chronic inflammation of neuronal cells and a loss of the BBB.

The microbiome in health and disease

Table 3 has shown the gut microbiome in healthy conditions. A metabolically healthy microbiota (mainly achieved by a high fiber, low animal fat and low protein diet and other aforementioned environmental factors) is shown in Figure 1. Microbial production of SCFAs provides an energy source for colonocytes and causes a decrease in luminal pH. The SCFAs acetate, butyrate and propionate can bind to G protein-coupled receptor (GPR) 41 and GPR 43, which are expressed on enteroendocrine L cells, and subsequently induce secretion of glucagon-like peptide 1 (GLP-1) and peptide YY (PYY), which contribute to increased energy expenditure, reduced food intake and improved glucose metabolism and insulin secretion (91). Butyrate is an activator of peroxisome proliferator-activated receptor-γ (PPARγ) and a stimulator of β-oxidation and oxygen consumption in the gut, which maintains an anaerobic environment in the gut lumen (92).

Pathobionts often responsible for dysbiosis are shown in Table 4. Figure 2 shows the metabolic pathways induced by gut dysbiosis. A dysbiotic microbiota is often associated with a prolonged colonic transit time, resulting in a shift in colonic metabolism leading to increased microbial proteolysis. Even though the preferred substrate for bacterial fermentation is fermentable dietary fibers, bacteria will not switch to protein metabolism until fermentable polysaccharides are depleted. As a result of increased protein fermentation, branched-chain fatty acids (BCFAs; 2-methylbutirate, isobutyrate and isovalerate), trimethylamine, organic acids, gases and trace amounts of phenols, amines, indoles and ammonia are produced, causing an increase in luminal pH (93). Altogether, such changes in the microbial environment and metabolites cause leakage of pathogen-associated molecular patterns (PAMPs), including lipopolysaccharides (LPS), which increase in the blood and trigger systemic

low-grade inflammation and insulin resistance (94). It should be noted, however, that some indole derivatives, such as 3-indolepropionic acid, produced by fermentation of dietary fibers have been shown to improve glucose metabolism (95).

Diseases associated or related to dysbiosis

Some diseases associated with gut microbiota abnormalities are shown in table 5.

Neurological diseases associated with gut dysbiosis have already been discussed.

Other diseases associated with gut dysbiosis are allergic diseases, inflammatory bowel syndromes or diseases, and metabolic diseases.

Allergic diseases

Gut microbiota dysbiosis is reported to be associated with allergic diseases such as eczema and asthma (96). Lower levels of gut *Staphylococcus aureus* and Clostridium and higher levels of Bifidobacterium are present in children affected by allergic symptoms (97). The cause has been ascribed to lipopolysaccharides that cause a reduced immune system response (98). The relationship of gut dysbiosis and asthma or other lung syndromes justifies the term "gut-lung axis" (99).

Irritable and inflammatory bowel syndromes

Irritable bowel syndrome (IBS) is a condition affecting 10% to 20% of adults, and children may also be affected. Bennet *et al* (100) described alterations in the gut microbiome of patients affected by IBS. Firmicutes are increased with a reduction in Ruminococcus and Bacteroides fragilis. The result is an excessive increase in SCFAs with consequent increase in serotonin that alters intestinal motility.

Inflammatory bowel disease (IBD) is a more severe condition. The most significant types of IBD are ulcerative colitis and Crohn's disease (101).

Several types of gut dysbiosis have been found in patients affected by IBD. Bacteroidetes and Firmicutes are decreased (102). Recently Zhu *et al* (103) found an increase in Proteobacteria and *Escherichia Coli*. One of the consequences of such dysbiosis is a reduction in mucus production that allows gut flora to pass more easily across the intestinal barrier, thus enhancing the inflammatory process (104).

Gut dysbiosis and metabolic diseases

The gut microbiota participates in material metabolism to produce metabolites. The gut microbiota can affect the metabolism of glucose, lipids and proteins by generating a series of metabolites and activating downstream signaling pathways as shown in figure 3.

Obesity

In addition to genetic and behavioral factors, the gut microbiome has an important role in the genesis of obesity (105).

Obese patient gut flora have higher levels of Firmicutes and lower levels of Bacteroidetes (106). Other bacteria found in the gut of obese patients are Bacteroides, Ruminococcus and Staphylococcus (107). These bacteria cause an increased degradation of β-glucuronide and aromatic amino acids, higher generation of organic acids and H_2 and higher biosynthesis of phenylalanine, tyrosine and tryptophan (108). In this condition, chronic inflammation is generated by the production of IL-1, tumor necrosis factor alpha (TNFα), monocyte chemoattractant protein-1 (MCP-1) and IL-6. In addition, lipopolysaccharides are produced that bind to the CD 14 receptor on the surface of immune cells to produce further inflammatory factors (109, 110).

Type 2 Diabetes Mellitus

Several studies have documented the influence of the gut microbiome on the pathophysiology of type 2 diabetes mellitus. In this condition, pathogenic flora prevail over protective flora.

Gurung *et al.* (111) analyzed 42 studies and found that the protective bacteria were Bifidobacterium and Bacteroides as well as Rosaburia and Faecalibacterium. In contrast, Ruminococcus, Fusobacterium and Blautia are pathobionts that, through the production of LPS, increase the permeability of the intestinal epithelium. In this condition, inflammatory molecules are produced, which increases insulin resistance (Figure 4). (112).

In contrast, protective flora produce IL-10 and other anti-inflammatory cytokines. In addition, protective flora exert their effect through the production of SCFAs and

butyrate (113). SCFAs also act as substrates for lipogenesis and gluconeogenesis in the liver.

Heart disease

Recent studies have also allowed finding a heart-gut axis. As in similar conditions, the effects of this axis are bivalent. Indeed, on one hand, heart failure induces an increase in permeability of the gut barrier (the so-called leaky gut) with consequent passage of gut microbiota and its products in the circulation inducing inflammation. On the other hand, several products of the gut microbiota, entering the blood may induce hypertension, atherosclerosis and heart failure.

The gut flora that is able to induce cardiovascular diseases is composed as follows (108). There is an increase in Enterobacteriaceae as *Escherichia Coli* and Klebsiella, and a decrease in Roseburia and Faecalibacterium prausnizii (114).

Several metabolic pathways are involved in the gut-heart axis: trimethylamine (TMA) and trimethylamine n-oxide (TMAO), SCFAs and bile acids (115).

Dietary sources including choline and l-carnitine provide substrates for microbiota-mediated production of trimethylamine. TMA after entering the portal circulation is converted by the hepatic host Flavin-containing monooxygenase (FMO) to TMAO. TMAO can promote atherogenesis and heart failure development. Adverse cardiac remodeling is also associated with elevated TMAO levels (116-118). A beneficial effect is exerted by SCFAs. SCFAs improve intestinal barrier function by promoting mucous production. In addition, they improve vascular tone through G protein-coupled receptor (GPCR) signaling. Finally, SCFAs activate histone acetyltransferase (HAT) and inhibit histone deacetylase (HDAC), thereby inhibiting inflammation and modulating immune cell activation (119).

Bile acids

Bile acids may be modified by the microbiome. They are produced by the liver, and a small portion is metabolized by colon microbiota with the production of secondary bile acids such as deoxycholate (DCA), lithocholate (LCA) and ursodeoxycholate (UDCA). These secondary bile acids are powerful agonists of farnesoid nuclear receptor (FXR),

which modulates metabolism and inflammation. Therefore, bile acids inhibit the anti-inflammatory activity of FXR. At the cardiovascular level, bile acids favor atherosclerotic disease, cardiac hypertrophy and hypertension (120).

Treatment and perspectives

Dysbiosis treatment is based on the use of prebiotics or probiotics. In addition, fecal microbiota transplantation has been proposed.

Prebiotics are dietary products that may change the composition and functions of microbiota by enhancing the presence of indigenous microbiota or by favoring the growth of specific bacteria.

Probiotics are live beneficial bacteria. Among these favorable effects to re-equilibrate, gut microbiota dysbiosis has been documented by Akkermansia muciniphila, Faecalibacterium prausnizii, Bacteroides uniformis, predator bacteria and phage therapy (8). Fecal microbiota transplantation is the process of transplantation of fecal microorganisms from healthy people to re-equilibrate gut microbiota dysbiosis.

In the future we can imagine the use of sequestrate or binding resins to eliminate harmful products or to sequestrate microbial metabolites. Other approach is the use of 3, 3-dimethyl-1-butanol (DMB) to reduce TMAO or TMA-lyase inhibitors.

Overall, several problems remain to be resolved.

There is substantial heterogeneity in the microbiota in both normal conditions and dysbiosis. In general, there is not a single bacterium but a collection of bacteria. This collection will likely not be best defined by the individual bacteria but rather by their metabolic capacities. This refers to the enzymes that are expressed, functioning and determining downstream metabolism.

The development of multiomics techniques, such as metagenomics, metabolomics and metatranscriptomes, should be further developed to answer these critical questions. Indeed, in addition to metagenomics the microbiome should also be analyzed by metabolomics studies in order to find its metabolic organization. Studies of genome-wide association will be used to correlate different genotypes with diseases phenotypes

and studies of metabolome wide association will correlate metabolic phenotypes with disease phenotypes.

89196_Auto_Edited.docx

ORIGINALITY REPORT

16%
SIMILARITY INDEX

PRIMARY SOURCES

1	curis.ku.dk Internet	171 words — 4%
2	www.zenodo.org Internet	98 words — 2%
3	pscpartners.org Internet	54 words — 1%
4	Yunyun Gao, Danyi Li, Yong-Xin Liu. "Microbiome research outlook: past, present, and future", Protein & Cell, 2023 Crossref	48 words — 1%
5	www.sochob.cl Internet	43 words — 1%
6	bsdwebstorage.blob.core.windows.net Internet	39 words — 1%
7	link.springer.com Internet	36 words — 1%
8	pubmed.ncbi.nlm.nih.gov Internet	31 words — 1%
9	Andrina Rutsch, Johan B. Kantsjö, Francesca Ronchi. "The Gut-Brain Axis: How Microbiota and Host	24 words — 1%

Inflammasome Influence Brain Physiology and Pathology", Frontiers in Immunology, 2020
Crossref

10	www.wjgnet.com Internet	24 words — 1%
11	www.ncbi.nlm.nih.gov Internet	21 words — 1%
12	academic.oup.com Internet	17 words — < 1%
13	Eman Zakaria Gomaa. "Human gut microbiota/microbiome in health and diseases: a review", Antonie van Leeuwenhoek, 2020 Crossref	15 words — < 1%
14	d-scholarship.pitt.edu Internet	14 words — < 1%

EXCLUDE QUOTES	ON	EXCLUDE SOURCES	OFF
EXCLUDE BIBLIOGRAPHY	ON	EXCLUDE MATCHES	< 12 WORDS